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Volume 39 *Number 7*

Phytopathology

AN INTERNATIONAL JOURNAL

July 1949

Official Organ of

The American Phytopathological Society

$7.75 per year, Canada $7.50 per year, United States and dependencies $8.00 per year, elsewhere

Office of Publication

Corner North Queen Street and McGovern Avenue, Lancaster, Pennsylvania

Entered at the postoffice in Lancaster, Pennsylvania, as second-class matter. Acceptance for mailing at the special rate of postage provided for in paragraph 4, sec. 538, P. L. and R. Authorised May 12, 1921.

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A NEW VIRUS DISEASE OF LADINO CLOVER[1]

K. W. KREITLOW AND W. C. PRICE[2]

(Accepted for publication February 23, 1949)

INTRODUCTION

A disorder of the Ladino variety of clover (*Trifolium repens* L.), characterized by chlorotic, yellow mottling of leaves, occurs extensively in the Northeastern United States (11). Severely affected plants frequently are stunted and bear distorted leaves. In the field, diseased plants occur individually or in spots one to several feet in diameter.

Plants of Ladino clover affected by the disease have been observed in central Pennsylvania since 1943, but some growers claim to have noted the disorder earlier. Diseased Ladino clover has either been seen in or received from the following States: Maine, Massachusetts, Vermont, Connecticut, Rhode Island, New Jersey, New York, Pennsylvania, Indiana, and Oregon.[3] More widespread culture of Ladino clover in recent years has undoubtedly increased the prevalence of the disease since it appears to be spreading and becoming more noticeable.

While no accurate estimates are available concerning the extent of damage caused by the disease, field observations indicate that it probably reduces yields by stunting the plants. Greenhouse observations show that affected plants are weakened and suggest that they are consequently more susceptible to adverse conditions such as drought and winter-injury.

The disease was readily reproduced by rubbing juice from infected plants onto leaves of healthy Ladino clover. Typical symptoms developed in from 12 to 21 days (Fig. 1, B). The causal agent is a virus, and these studies on its properties and on transmission demonstrate that the virus is a strain of alfalfa mosaic virus different from any previously described. The disease in Ladino clover is referred to as yellow patch and the virus is given the trinomial *Marmor medicaginis* H. var. *Ladino* n. var.

LITERATURE

Viruses infecting *Trifolium repens* L. in the field have been described by several authors. A number of additional viruses have been artificially transmitted to white clover. Zaumeyer and Wade (20, 21, 22) described a virus disease of *T. repens* that caused both systemic and local infection

[1] Contribution No. 91 of the U. S. Regional Pasture Research Laboratory, Division of Forage Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, U. S. Department of Agriculture, State College, Pennsylvania, in cooperation with the Northeastern States.

Aided by a grant from the National Foundation for Infantile Paralysis.

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[3] In a personal communication, Dr. E. A. Hollowell states that he has seen and collected specimens of the disease in Alabama, North Carolina, Ohio, Wisconsin, and Iowa.

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when transmitted to *Phaseolus vulgaris* L. Pierce (14) recovered from white clover a virus that infected *P. vulgaris* and several other species of legumes, but failed to infect soybean or tobacco. In a later test, Pierce (15) identified white clover virus 1 in five collections of diseased *T. repens*. Valleau (18) reported a virus disease of white clover, characterized by

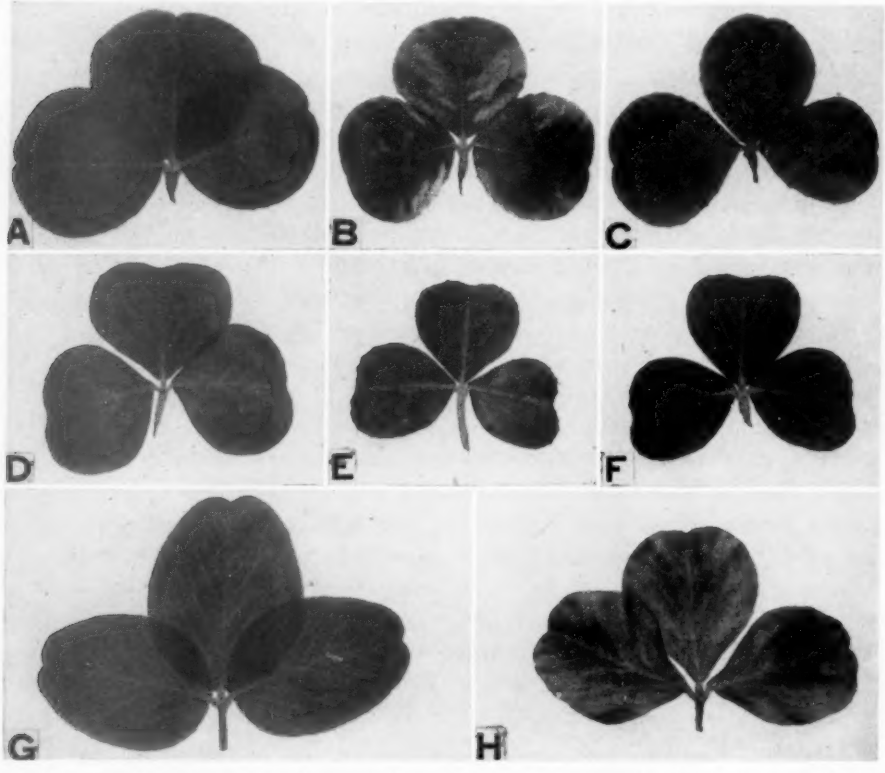

FIG. 1. Symptoms of yellow patch and white clover mosaic viruses in several species of *Trifolium*. Ladino clover: A. Healthy leaf. B. Leaf with yellow patch. C. Leaf with white clover mosaic. *T. incarnatum:* D. Healthy leaf. E. Leaf with yellow patch. F. Leaf with white clover mosaic virus. *T. pratense:* G. Healthy leaf. H. Leaf with yellow patch.

yellowish patches on leaves. The virus was transmitted mechanically to bean and tobacco. E. M. Johnson (10) isolated the same or a similar virus from *T. repens* which he transmitted to several hosts including tobacco. The virus produced local lesions on bean but failed to infect alfalfa, white clover, zinnia, or pepper. F. Johnson (8, 9) showed that a disease of white clover previously regarded as caused by a single virus, classified by Weiss (19) as Trifolium virus 1, was caused in reality by a mixture of two viruses. The viruses could be separated because dodder picked up and transmitted only one of the constituents and *Vigna sinensis* (Torner) Savi. was susceptible only to the other.

MATERIALS AND METHODS

Most of the virus material used in the tests was obtained from naturally infected plants of Ladino clover collected in the vicinity of State College, Pennsylvania. Seedlings of Ladino clover and other hosts tested were grown during the winter in flats or pots of steamed soil in a greenhouse that was fumigated regularly.

Healthy seedlings were inoculated by rubbing a square of sterile gauze dipped in juice of diseased plants over leaves previously dusted with carborundum powder. The leaves were then sprinkled with tap water and the plants were incubated in a greenhouse at 18–20° C. Controls were inoculated with juice from healthy plants.

For comparative purposes, most hosts tested were also inoculated with a white clover mosaic virus previously used by Atwood and Kreitlow (2) and maintained in a greenhouse in plants of Ladino clover. This virus produced symptoms resembling those produced by the white clover mosaic virus described by Johnson (8, 9).

SYMPTOMS

Symptoms in plants of Ladino clover inoculated in the greenhouse with yellow patch virus varied, depending upon susceptibility of the individual plant and on environmental conditions prevailing during the incubation period. In mildly affected plants, earliest symptoms developed in from 12 to 21 days and consisted of clearing of veins and mottling in newly developed leaves. In older leaves, the mottling gradually gave place to a condition in which yellowish areas appeared between veins and toward the margins of the leaf. In some cases, only a few small yellow areas ultimately developed in affected leaves and the plants usually showed no other visible damage.

In plants severely affected by yellow patch, earliest symptoms appeared in from 12 to 15 days. New leaves that unfolded were chlorotic and distorted. Later, green areas of unaffected leaf tissue delimited the somewhat angular, yellow patches characteristic of the disease. Necrotic areas occasionally developed in leaves of plants severely attacked. Stolon internodes frequently were greatly shortened and leaf petioles were stunted. Severely affected plants grew slowly, were weakened, and frequently failed to survive short periods of high temperature or excessive drying.

Symptoms in most affected plants were masked at high temperature. They developed most strikingly at 18–24° C. They tended to disappear during long periods of cloudy weather.

HOST RANGE

A number of species of plants were tested for susceptibility to yellow patch virus. Results of these tests are summarized in table 1. Symptoms produced by the virus in several hosts are illustrated in figures 1 and 2.

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TABLE 1.—*Comparative host range of yellow patch virus and white clover mosaic virus*

Family and species	Number of plants infected[a]		Symptoms[b] of	
	Yellow patch virus	White clover virus	Yellow patch virus	White clover virus
Amaranthaceae				
Celosia argentea L. var. *cristata* Kuntze	2/2	.....	L—SP—CL	.....
Apocynaceae				
Vinca rosea L.	10/10[c]	.....	S—VC, SP—CL	.....
Balsaminaceae				
Impatiens holstii Engler and Warb.	2/2	.....	S—SP—CL	.....
Chenopodiaceae				
Beta vulgaris L.	0/10	0/10	None	None
Spinacea oleracea L.	0/10	0/10	do	do
Compositae				
Callistephus chinensis Nees.	0/10	0/10	do	do
Lactuca sativa L.	0/10	0/10	do	do
Zinnia elegans Jacq.	9/10[c]	0/10	S—ST—C—CL	do
Cucurbitaceae				
Cucumis sativus L.	0/10	0/10	None	do
Leguminosae				
Glycine max (L.) Merr.	9/10[c]	0/10	S—ST—V, C, P—R	do
Lathyrus odoratus L.	5/8[d]	7/8	S—W—C—CL or L—SP—CL, N	S—M, VC
Lotus corniculatus L.	0/10	0/10	None	None
Lupinus sp.	0/2	.....	do	.....
Medicago arabica (L.) All.	3/10[c]	0/10	S—ST—M, C—CL	S—M, VC
M. hispida Gaertn.	1/10[d]	2/10	S—M, VC—CL	S—VC
M. lupulina L.	0/3	0/3	None	None
M. obscura Retz.	3/3[c]	3/3	S—M, VC, C—CL	S—VC, C
M. orbicularis (L.) All.	1/2[c]	.....	S—ST—M, VC, D—CL	.....
M. sativa L.				
Atlantic	0/10	0/10	None	None
Buffalo	0/10	0/10	do	do
Grimm	0/30	0/30	do	do
Ranger	0/10	0/10	do	do
Melilotus alba Desr.	8/10[d]	6/10	S—W—M, C—CL	S—M, VC, C
M. indica (L.) All.	6/10[c]	2/10	S—ST—M, VC, C—CL	S—M, VC, C
M. officinalis (L.) Lam.	2/3[d]	2/3	S—M—CL	S—M, VC
M. suavcolens Ledeb.	10/10[d]	8/10	S—K—M, VC, C—CL	S—ST—VC, C
Phascolus lunatus L.	8/8[c]	0/8	S—M—CL	None

Family and species	Number of plants infected[a]		Symptoms[b] of	
	Yellow patch virus	White clover virus	Yellow patch virus	White clover virus

Family and species	Number of plants infected[a]		Symptoms[b] of	
	Yellow patch virus	White clover virus	Yellow patch virus	White clover virus
Phaseolus vulgaris L.				
Sure Crop Wax	18/19	0/17	L—SP—R	None
Northrup King Tendergreen	10/10	0/10		
Plentiful	8/8	0/8		
Burpee Stringless greenpod	9/9	0/9		
Woodruff Full Measure	9/9	0/9		
Bountiful	10/10	0/10		
Pisum sativum L.				
Marvel	24/26[c]	12/12	S—ST—M, VC, C—CL	S—M, VC, C
Potlatch	25/28	11/14		
Excelsior	22/26	13/14		
Blue Bantam	25/30	10/15		
Trifolium alexandrinum L.	2/10[d]	6/10	S—ST—M, VC—CL	S—VC, C
T. dubium Sibth.	4/10[d]	1/10	S—M, VC—CL	S—VC, C
T. fragiferum L.	6/10[c]	0/10	S—M, VC, C—CL	None
T. glomeratum L.	2/10[d]	1/10	S—M, VC, D—CL	S—M, VC, C
T. hirtum All.	7/10[c]	4/10	S—ST—C, D—N	S—ST—VC
T. hybridum L.	9/10[c]	5/10	S—ST—M, VC, C, D—CL	S—VC, C
T. incarnatum L.	10/10[c]	6/10	S—ST—M, V, C—B	S—VC, C
T. pratense L.	+[c]	+	S—ST—M, D—CL	S—M, VC, C
T. procumbens L.	0/10	0/10	None	None
T. repens L. (Ladino)	+[c]	+	S—ST—M, VC, D—CL	S—M, VC, C
T. resupinatum L.	10/10[c]	9/10	S—ST—M, VC, C, D—CL	S—ST—M. VC, C
T. subterranean L.	9/10[c]	5/10	S—ST—M, VC—CL	S—ST—VC, C
Vigna sinensis (Torner) Savi.	5?/6[d]	0/6	L—V, SP—N, R	None
Scrophulariaceae				
Antirrhinum majus L.	7/10[c]	0/10	S—ST—VC, D—CL	None
Solanaceae				
Capsicum frutescens L.	9/10[c]	0/10	S—M, D—CL	None
Lycopersicon esculentum Mill.	0/10	0/10	None	None
Nicotiana glutinosa L.	2/2[c]	.....	S—ST—M—CL or L—SP—CL	None
N. rustica L.	2/2[c]	.....	S—ST—M, SP—CL	None
N. tabacum L.	10/10[c]	0/10	S—ST—M, P, D—CL	None
Petunia hybrida Vilm.	10/10[c]	0/10	S—ST—M, VC, C, D—CL	None

[a] The numerator indicates number of plants infected; the denominator, number of plants inoculated.

[b] Symptoms are designated by symbols:

Infection was local (L) or systemic (S).

Plants were stunted (ST), wilted (W), or killed (K) by the virus.

Leaf symptoms were mottling (M), vein discoloration (V), vein clearing (VC), downward curling (C), puckering (P), distortion (D), or spotting (SP).

Tissues affected were chlorotic (CL) or necrotic (N); red (R) or brown (B).

[c] The virus was recovered.

[d] The virus could not be recovered.

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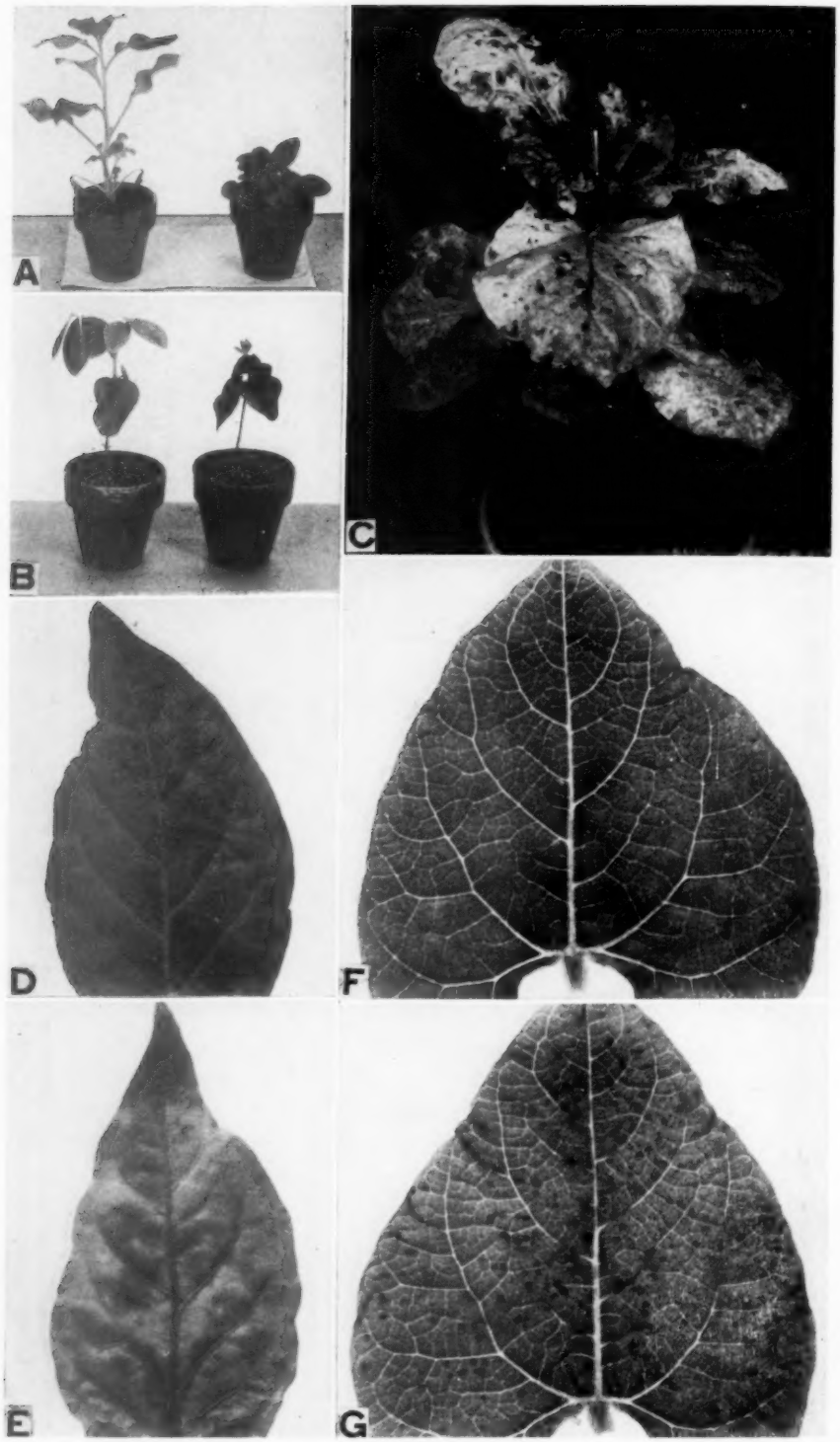
A
B
C
D
F
E
G

Yellow patch virus has a much broader host range than white clover mosaic virus. In common hosts, symptoms were usually distinctive. In most cases, symptoms produced by white clover mosaic virus were less severe than those produced by the yellow patch virus.

Symptoms produced by yellow patch virus were milder in some hosts than in others. Infected leaves of *Zinnia elegans* Jacq. and *Antirrhinum majus* L. developed a greenish-yellow chlorosis instead of the bright yellow chlorosis observed in other hosts. Leaves of zinnia curled downward only slightly and leaves of snapdragon twisted instead of curling downward.

Infected plants of *Glycine max* (L.) Merr. developed a deep green color that was maintained long after control plants matured and turned yellow.

Inoculated leaves of peas infected with yellow patch virus died prematurely. Plants with severe symptoms had greatly shortened internodes and produced leaves in tightly bunched clusters.

Symptoms of yellow patch virus in Turkish tobacco were similar to those caused by a strain of alfalfa mosaic virus isolated some years ago from a yellow spot that developed on a tobacco leaf infected with the type strain. Symptoms caused by the mutant strain are illustrated in figure 2, C.

Local lesions that developed on sweet pea were parchment-like and measured 3–5 mm. in diameter. They eventually coalesced so that infected leaves turned yellow, wilted, and dropped. Later, the terminal bud wilted and the plant died. When lateral branches developed before the plant died, they bore leaves that were small and curled.

The fact that yellow patch virus produced local lesions on bean afforded a convenient method of testing for the presence of virus in other hosts. Juice from leaves of hosts with symptoms of infection, or from hosts suspected of harboring the virus in a latent state, was used to subinoculate leaves of bean seedlings of the variety Bountiful. When local lesions measuring 1–2 mm. in diameter developed within 48–72 hours, the source host tested was considered infected with the yellow patch virus whether symptoms were evident or not.

Although several varieties of alfalfa (*Medicago sativa* L.) were inoculated, no infection was obtained in this host. No symptoms resulted from the inoculation, and virus could not be recovered by rubbing juice of inoculated alfalfa onto leaves of bean seedlings at frequent intervals following inoculation. Other species of *Medicago* varied in susceptibility to infection. Both *M. arabica* (L.) All. and *M. obscura* Retz. were susceptible

FIG. 2. Symptoms of yellow patch virus produced in different hosts. A. (Right) Severe stunting in petunia (healthy plant at left). B. (Right) Stunting and curling of leaves in soybean (healthy plant at left). C. Chlorotic mottling in Turkish tobacco produced by a mutant isolated from the type strain of alfalfa mosaic virus. The symptoms are identical with those produced by the yellow patch virus. D. Leaf of healthy pepper plant. E. Chlorosis in pepper leaf infected with yellow patch virus. F. Leaf of healthy Bountiful bean. G. Local lesions produced in Bountiful bean by yellow patch virus.

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while *M. lupulina* L. and *M. hispida* Gaertn. resisted infection. *M. obscura* also was readily infected by white clover mosaic virus. Several species developed virus-like symptoms following inoculation with the yellow patch virus but local lesions failed to appear when juice from these plants was sub-inoculated to leaves of bean seedlings.

In general, most species of Leguminosae tested were susceptible to yellow patch virus. Other susceptible species were found in the Compositae, Scrophulariaceae, Solanaceae, Apocynaceae, Amaranthaceae, and Balsaminaceae.

TESTS WITH SINGLE-LESION STRAINS OF THE VIRUS

Since Johnson (8, 9) showed that white clover mosaic is caused by a mixture of two viruses, a test was made to determine whether the yellow-patch disease was caused by a single virus or by a mixture of one or more viruses. A dilute suspension of the virus from tobacco was rubbed over Bountiful bean leaves. Single, well-isolated, local lesions that developed were punched out, macerated in a few drops of water, and rubbed over leaves of pepper. The symptoms produced in pepper by these single-lesion transfers were identical with those obtained previously in mass inoculations. Juice from infected peppers was sub-inoculated to tobacco. Typical symptoms of yellow patch developed. In a similar test, a single-lesion strain of the virus was carried from bean to tobacco, to periwinkle, then back to Ladino clover. Again, typical symptoms developed. These tests suggest that the disease in Ladino clover is caused by a single virus entity, not a complex.

ATTEMPTS TO TRANSMIT THE VIRUS WITH DODDER

Johnson (8, 9) found that one member of the white clover mosaic virus complex could be transmitted by dodder, *Cuscuta campestris* Yunck. To determine whether or not dodder could transmit yellow patch virus, strands parasitizing plants of Ladino clover infected with the virus were trained to healthy seedlings of Ladino clover. Despite connection of healthy and diseased plants for 6–8 weeks, no symptoms developed. When juice from seedlings of Ladino clover connected by dodder to infected plants was rubbed onto leaves of Bountiful bean, no local lesions or other symptoms of virus infection developed. Juice was then extracted from dodder parasitizing virus-infected plants and rubbed onto leaves of bean. No local lesions or other symptoms developed. These tests indicate that the yellow patch virus is not readily picked up or transmitted by dodder.

PROPERTIES OF THE VIRUS

To determine the thermal inactivation point for yellow patch virus, juices from infected plants of Ladino clover and of Turkish tobacco were heated in a water bath for 10 min. at various temperatures. Activity of the heated samples was determined by inoculation of from 4 to 24 primary

leaves of Bountiful bean seedlings. The data obtained indicate that the thermal inactivation point lies between 62° and 63° C.

In similar tests, solutions of the virus buffered at pH 7.0 were incubated at 18°–20° C. At intervals samples were removed and inoculated onto leaves of Bountiful bean seedlings. They produced from 25.7 lesions/sq. cm. at 0 hours to 3.1 lesions/sq. cm. after 48 hours. At 28° C., the number of lesions produced on Bountiful bean was reduced 80 per cent in 60 minutes.

Virus activity was lost when the juice from infected Ladino clover plants was diluted beyond 1:100.

IDENTIFICATION OF YELLOW PATCH VIRUS

From symptomatology, host range, and virus properties, it is apparent that the yellow patch virus has much in common with strains of alfalfa mosaic virus. The virus produced local lesions on bean and mottling in tobacco resembling symptoms produced by alfalfa mosaic. According to Price (16), alfalfa mosaic virus infects a wide range of hosts. The yellow patch virus was capable of infecting hosts in 6 of the 9 families tested. Most of these hosts are known to be susceptible to alfalfa mosaic virus (6, 16, 19). Moreover, the thermostability of yellow patch virus is comparable to that of the type strain of alfalfa mosaic virus.

The yellow patch virus differs from other strains of alfalfa mosaic virus in several respects. Symptoms on tobacco distinguish it from the type strain, from the three strains studied by Zaumeyer (23), and from the strain isolated from pepper by Berkeley (3). Its tolerance to dilution and aging-in-vitro distinguish it from the strain reported by Pierce (13) and the strain that attacks celery reported by Snyder and Rich (17). Although symptoms in *Trifolium repens* produced by the yellow patch virus resemble those produced by a virus obtained from white clover by Zaumeyer and Wade (20, 21, 22), other properties of the two viruses are dissimilar. The strain of alfalfa mosaic virus from *T. repens* described by Valleau (18) and Johnson (10) possessed many of the characteristics of the yellow patch virus, but the thermal death point of Johnson's isolate was lower (55°–58° C.). Moreover, Johnson was unable to transmit his virus to white clover, zinnia, or pepper which were readily infected by the yellow patch virus. Ainsworth and Ogilvie (1) reported a virus-like disorder of white clover but were unable to transmit it to *T. repens* or other hosts tested.

Several additional viruses have been reported capable of infecting *Trifolium repens* L. but none of these are similar to the yellow patch virus. Although potato calico virus (4) infects many of the same hosts as the Ladino clover virus, the calico virus infects *Cucumis sativus* L. and yellow patch virus does not. Other properties of the two viruses also are dissimilar. Dickson (5) and Osborn (12) reported artificial infection of *T. repens* by a mosaic virus of red clover but failed to transmit the virus

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to bean or tobacco. Johnson and Jones (7) infected *T. repens* with pea severe mosaic virus but were unable to transmit the virus to tobacco. Infectivity to tobacco distinguishes the yellow patch virus from several other virus diseases of *T. repens* (8, 9, 14).

On the basis of differences here reported it is concluded that the yellow patch virus is a strain of alfalfa mosaic different from any described previously.

Technical description

Marmor medicaginis H. var. **Ladino** n. var.

Common name: Ladino clover yellow patch virus. Differing from the type strain in symptomatology on tobacco and tolerance to dilution and aging-in-vitro.

Hosts: LEGUMINOSAE—*Trifolium repens* L. var. *Ladino*, Ladino clover. Also transmissible experimentally to many species of dicotyledonous plants including AMARANTHACEAE—*Celosia argentea* L. var. *cristata* Kuntze, cockscomb. APOCYNACEAE—*Vinca rosea* L., periwinkle. BALSAMINACEAE—*Impatiens holstii* Engler and Warb., balsam. COMPOSITAE—*Zinnia elegans* Jacq., zinnia. LEGUMINOSAE—*Glycine max* (L.) Merr., soybean, *Phaseolus vulgaris* L., bean, *Pisum sativum* L., pea. SCROPHULARIACEAE—*Antirrhinum majus* L., snapdragon. SOLANACEAE—*Capsicum frutescens* L., pepper, *Nicotiana tabacum* L., tobacco.

Geographical distribution: United States.

Induced disease: In Ladino clover, tobacco, pepper, and periwinkle, systemic chlorotic yellow mottling, occurring mostly in patches. In varieties of garden bean, numerous reddish necrotic local lesions.

Transmission: By inoculation of expressed juice in dilutions up to 1:100. Not by dodder, *Cuscuta campestris* Yunck.

Thermal inactivation: At 62°–63° C. in 10 min., at 18°–20° C. in 48 hr.

DISCUSSION

Cross-protection tests with the type strain or with other strains of alfalfa mosaic virus would have aided in establishing the relationship of yellow patch virus to alfalfa mosaic virus, but none of these strains could be obtained for comparison. Despite this, the host range and virus properties demonstrate the close relationship of yellow patch virus to other described strains of alfalfa mosaic virus. The broad host range suggests that yellow patch virus may possibly be transmitted from other hosts to Ladino clover by an unknown insect vector. Seeds harvested from plants of Ladino clover infected with yellow patch virus have yielded disease-free seedlings. This indicates that the yellow patch virus is probably not seed-transmitted in Ladino clover.

Since winter survival is a major problem in growing Ladino clover and observations indicate that plants infected with yellow patch virus are weakened, there is a further possibility that diseased plants may succumb

to winter injury ordinarily not damaging to healthy plants. The increasing importance of Ladino clover as a forage legume and the corresponding increase in prevalence of yellow patch virus in this host suggest that measures for controlling or eliminating the disease should be developed.

SUMMARY

A disorder of Ladino clover referred to as yellow patch and characterized by chlorotic, yellow mottling of leaves is caused by a virus. Symptoms of the disorder were readily reproduced on a number of different hosts by rubbing juice from diseased plants of Ladino clover onto leaves of healthy plants. Tests with single-lesion strains of the virus demonstrated that the disease was caused by a single virus entity. The virus was not transmitted or taken up by dodder.

Virus activity was lost when juice from infected plants of Ladino clover was diluted beyond 1:100. A solution of the virus permitted to stand at 18°–20° C. was inactive after 48 hr. The thermal inactivation point for the virus was 62°–63° C.

From the host range and other properties, the virus was identified as a strain of alfalfa mosaic virus different from any described previously. The virus was given the trinomial *Marmor medicaginis* H. var. *Ladino* n. var.

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LABORATORY AND GREENHOUSE STUDIES OF ANTIMYCIN PREPARATIONS AS PROTECTANT FUNGICIDES[1]

CURT LEBEN AND G. W. KEITT[2]

(Accepted for publication February 24, 1949)

With the remarkable successes in the use of antibiotics in the control of diseases of animals, there has been an increase in interest in the possibilities of use of such materials against plant diseases. A number of antibiotics have been employed in seed treatments (3, 4, 5, 9, 22, 23), in inducing artificial resistance to disease (3, 4), and in chemotherapy (4, 6, 7, 10, 20), with varying results. This laboratory has published preliminary reports of an antibiotic as a protectant fungicide under greenhouse conditions (15, 16). The purpose of the present paper is to report these and further investigations of this antibiotic.

MATERIALS AND METHODS

Antibiotic preparations. The antibiotic material treated in this report is produced by an unidentified species of *Streptomyces.* In a previous study (17) the production, properties, and biological activities of crude preparations were considered, and the name antimycin was proposed for the active material. Later (8) an active crystalline compound was isolated from these crude preparations. The name antimycin A was then proposed for this crystalline substance. There is evidence that the crude material contains one or more antibiotic factors in addition to antimycin A.

The preparations used in the present studies were derived from shake or tank cultures (8) of the antibiotic organism. Their antibiotic potency, in terms of units per ml., was determined by a plate assay using *Glomerella cingulata* (Stoneman) Spauld. and v. Schrenk as the assay organism (8). The active material was extracted with ethanol from the precipitate formed on the acidification of the culture filtrate. In the early experiments in the greenhouse this ethanol extract, which will be known hereinafter as "crude antimycin in ethanol," was used as a protectant spray. Later, aqueous suspensions were employed. These were made by removing the ethanol over water by reduced pressure distillation. The active material remained in the water as an insoluble, green-brown, fine precipitate that settled slowly. Unless otherwise noted these suspensions were used in all experiments reported in this paper; they are designated as "crude antimycin suspensions" or more simply as "suspensions." Their color, texture, rate of settling, and *p*H (usually 4.0 to 5.0) varied from fermentation to fermentation. Purity also varied: suspensions from seven to 58 units per mg.

[1] Published with the approval of the Director of the Wisconsin Agricultural Experiment Station. Supported in part by a grant from the Schenley Research Institute.

[2] Research associate and professor, respectively. The authors are indebted to F. M. Strong, B. R. Dunshee, G. J. Stessel, W. H. Peterson, and M. S. Johnson for aid in various phases of the problem, and to Eugene Herrling, who prepared the photographs.

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(dry weight) were employed in the various studies. Crystalline antimycin A (*ca.* 1000 units per mg.) was used in the form of a microsuspension in water.

Foliage disease methods. In part of the work two foliage diseases, apple scab and tomato early blight, were used in the evaluation of the various antibiotic preparations under greenhouse conditions. Ordinarily, susceptible leaves were sprayed with test material, dried (usually 2–4 hr.), and inoculated with a spore suspension of the disease-inciting fungus, and the plants were placed under conditions favorable for the development of the disease. In some tests plants were washed with simulated rain after the antibiotic spray had dried. These plants were then allowed to dry before inoculation. In the incitation of apple scab, the methods of Keitt

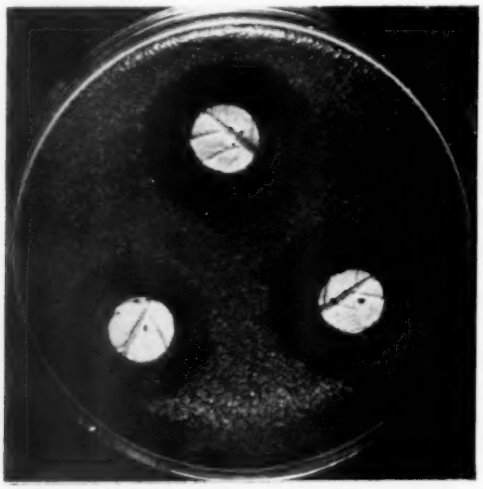

FIG. 1. Inhibition zones produced by leaf disks sprayed with crude antimycin suspensions.

and Langford (13) were used; in the incitation of early blight, those of McCallan and Wellman (19) were employed with certain exceptions.[3] Precision spray apparatus was not available; in all of the studies reported in this paper antibiotic preparation and spore suspensions were sprayed on the upper sides of the leaves, as uniformly as possible by hand, with a DeVilbiss No. 15 atomizer. Spraying was stopped when runoff was about to begin.

Leaf disk assay. A preliminary report on the use of this assay for determining the amount of antibiotic activity on leaves has appeared (14). The method depends on the diffusion of the antibiotic from treated leaf disks into an agar medium containing an assay fungus. The amount of antibiotic on the disks is estimated by comparing the resulting inhibition

[3] An isolate of *Alternaria solani* (E. and M.) Jones and Grout requiring no special treatment (18, 19) for the production of spores was used. In different series of experiments, plants were 5–8 in. high, and, depending on the series, lesions on 3 or 4 leaves were counted.

zones with those produced by known amounts of the antibiotic in blotting paper disks. Ordinarily plants were sprayed with the antibiotic preparations, dried (2–4 hr.), and leaf disk samples taken at random. In rain tests, additional samples were taken after the plants had been subjected to simulated rain and dried.

Problems of contamination were encountered in the development of the leaf disk assay. When leaf disks treated with crude antimycin suspensions replaced the blotting-paper disks in the regular assay (8), fast-growing bacteria from the disks usually obscured the inhibition zones. This difficulty was overcome by adjusting the assay medium from *p*H 6.8 to 4.0; however, under these conditions the edges of the zones were too indistinct to be measured accurately. Consequently, studies were made with various acidic media and a number of fungi that had been shown previously to be inhibited by the crude material. Ultimately a combination that gave suitable zones was selected (Fig. 1). Under the conditions of this work growth of bacteria was controlled and only occasionally did fast-growing fungi from the disks prevent measurement of zones. The assay procedure follows.

Fifteen ml. amounts of acidified potato-dextrose agar[4] were allowed to harden in a series of Petri plates. These were then layered with 5 ml. of an agar spore suspension, which was made by mixing one volume of a distilled water spore suspension of *Colletotrichum circinans* (Berk) Vogl.[5] with 4 volumes of acidified potato-dextrose agar at 38–40° C. When the suspension had solidified, leaf disks 0.5-in. in diameter were cut from treated plants and placed, sprayed side downward, on the surface of the agar. Each disk was pressed gently to insure complete contact. At the same time blotting-paper disks 0.5-in. in diameter containing different ethanol dilutions of a standard solution of crude antimycin in ethanol were also placed on the seeded medium. Disks from four treatments usually were put on one plate. Each treatment was replicated six times, each time on a different plate. Plates were incubated 40–48 hr. at 28° C. and the average diameter of the inhibition zones was determined. A curve was plotted with the log of the antibiotic potency of the standard as the abscissa and the diameter of the inhibition zone as the ordinate. An example of a standard curve is given in figure 2. The same curve was produced when the standard was tested at the beginning or at the end of the time (1–3 hr.) the leaf disks were being placed on the agar. The amount of antibiotic on the leaf disks was determined by interpolation (sometimes extrapolation) of the standard curve and expressed in "leaf units." One leaf unit is the amount of active material on a leaf disk that will produce an inhibition zone the same size as that produced by a blotting paper disk containing 1 unit per ml. of the standard. To standardize further the term "leaf unit,"

[4] Per liter: extract from 200 gm. of sliced, peeled potatoes steamed 0.5 hr. in 500 ml. of water; glucose, 25 gm.; agar, 17 gm. The reaction was adjusted with 2N HCl immediately before the plates were poured so that the *p*H of the medium was 3.8–4.2.

[5] Our isolate 30. The water suspension contained about 100,000 spores per ml. Spores were removed from potato-dextrose-agar slants 7–14 days after seeding.

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it will be necessary to specify the volume of the standard added to the blotting-paper disks; in the present studies one lot of blotting paper was used in making the disks, and they were all dipped and drained in a uniform manner.

Accuracy of the leaf disk assay is indicated by the average and standard deviation determined from several tests: 10.9 ± 1.6 (4 replicates), 40.5 ± 6.5 (6 replicates), 18.3 ± 1.4 (8 replicates), 26.1 ± 4.0, 11.5 ± 1.6, and 10.3 ± 1.6 (all with 10 replicates) leaf units. It is probable that accuracy can be increased by further study.

The leaf disk assay appears to be advantageous because of its relative

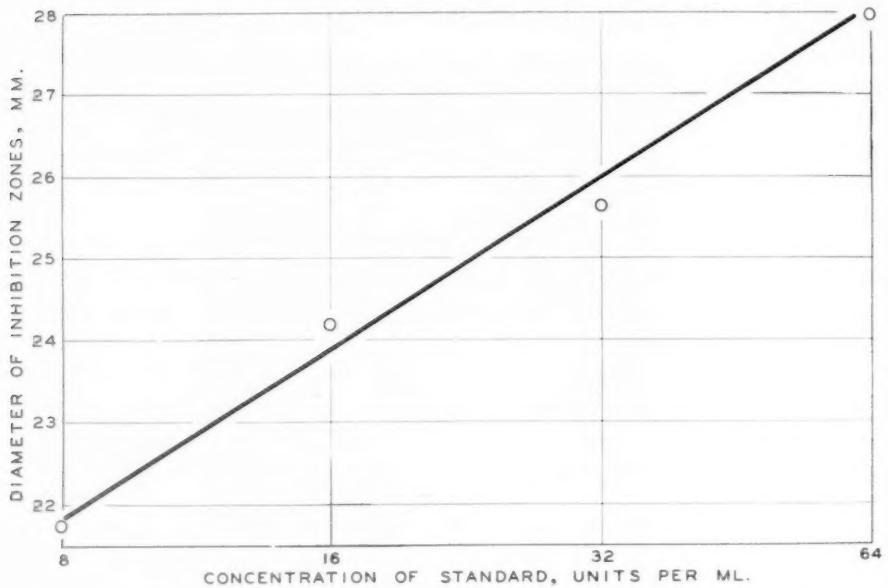

FIG. 2. The relation of the concentration of antibiotic in blotting-paper disks to inhibition zone size.

simplicity and because fungicide-plant interactions are taken into consideration. Preliminary studies indicate that with suitable modification the assay may be used for diffusible fungicides not of microbial origin. Leaves of tomato (*Lycopersicon esculentum* Mill.), apple (*Malus sylvestris* Mill.), cowpea (*Vigna sinensis* (L.) Endl.), elm (*Ulmus americana* L.), and *Lonicera tatarica* L. have been used in leaf disk assays. With these species no inhibition zones were produced unless leaves were first sprayed with a suitable fungicide.

Simulated rain tests. Simulated rain was produced by lake water flowing through a flat type hose sprinkler, which was directed slightly upward so that the water fell gently by gravity on one side of an electrically driven turntable. Test plants were placed toward the edge of the turntable so that on each revolution each plant passed through a zone of simu-

lated rain. The turntable, which was 4 ft. in diameter, made 4 revolutions per min.; the water fall per minute on any given plant was approximately 1 in.

Slide-germination methods. A precision horizontal sprayer modeled after that described by Horsfall (11) was used to note the inhibiting effects of various antimycin preparations on spores of *Glomerella cingulata*. Routine methods (1) were used. Tests were made at 28° C., and filtered orange juice was employed as a stimulant.

EXPERIMENTAL RESULTS

The protective effect. Crude antimycin in ethanol was first shown to be effective as a protectant in greenhouse tests with *Venturia inaequalis* (Cke.) Wint. The results of typical experiments are given in table 1.

TABLE 1.—*The effect of crude antimycin in ethanol on infection of apple leaves by Venturia inaequalis*

Experiment number	Test shoots[a]		Control shoots[c]	
	No.	Symptoms[b]	No.	Symptoms
1	8	4 flecks	6	150 lesions
2	6	none	6	150 do
3	10	2 lesions, 6 flecks	8	1000 do

[a] Shoots sprayed with antibiotic solutions. Shoots in experiment 1 sprayed with one preparation (1 unit per ml.) and shoots in experiments 2 and 3 sprayed with another preparation (2 units per ml.).

[b] Lesion is the typical symptom; fleck is a reduced chlorotic area, usually with no sporulation.

[c] Sprayed with ethanol.

Under some conditions a marked distortion or killing of the upper leaves was noted, caused by the ethanol in which the antibiotic was carried. Water suspensions or 25 per cent ethanol suspensions did not induce this injury when used in the control of apple scab or early blight (Table 2).

TABLE 2.—*The effect of a crude antimycin suspension on the control of tomato early blight*

Potency of spray in units per ml.	Lesions per plant[a]	
	Test 1	Test 2
No.	No.	No.
12	0	0
3	0	0
0	69	61

[a] Single plant tests except for controls. Control for test 1, average of 3 plants; for test 2, average of 2 plants.

Consequently, aqueous suspensions were used in subsequent experiments.

In later studies a dosage-response curve was plotted for early blight (Fig. 3).

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Deposition. In a series of studies with the leaf disk assay, several factors were found to influence the amount of antibiotic deposited. The deposit varied with different species of plant. For example, the leaf unit values produced by one antibiotic suspension with tomato, cowpea, *Lonicera tatarica*, and elm were, respectively, 16.5, 9.3, 9.8, and 8.0. Differences in the deposit were noted also with various suspensions although they were

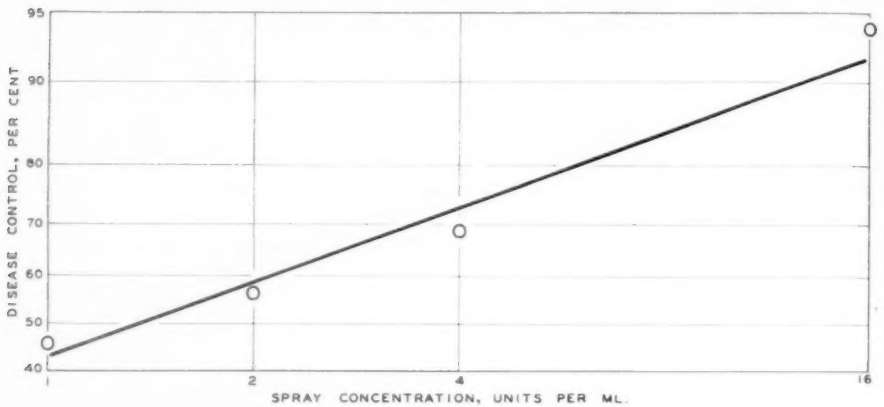

FIG. 3. Dosage-response curve for a crude antimycin suspension against tomato early blight. Single plant tests were repeated in experiments completed at different times. Each point is the average of 3 tests, except at 2 units per ml., where there were 2 tests.

of approximately the same antibiotic potency. Certain insecticides, surface-active compounds, or stickers also influenced the amount of antibiotic retained on the leaf. Probably other factors such as humidity, temperature, and age and condition of the plant are also important.

TABLE 3.—*Effect of different time intervals between spraying and inoculation on the control of apple scab by a crude antimycin suspension*

Hours spray applied before inoc.	Spray material[a]	Lesions per shoot[b]	Disease control
No.		No.	Per cent
96	Test	13.0	78.4
Do	Control	60.1	
48	Test	1.8	95.7
Do	Control	42.5	
4	Test	0.0	100.0
Do	Control	81.0	

[a] Test spray: 3 units per ml. carried in 25 per cent ethanol; control spray: 25 per cent ethanol.

[b] Average of 10 test shoots or 4 control shoots.

Stability on plant leaves. Greenhouse studies with *Venturia inaequalis* indicated that one antibiotic suspension lost some activity after 96 hr. on apple leaves (Table 3). With another suspension, a loss in activity on tomato and apple leaves was noted (Table 4): approximately one half of the original activity remained after 4 days. On tomato leaves part of

TABLE 4.—*The stability of crude antimycin on plant leaves*

Test[a]	Activity in leaf units on leaves after:		
	4–6 hours	2 days	4 days
	No.	No.	No.
1	13.0	9.5	6.2
2	15.0	12.4	7.2
3	12.4	12.0	7.5

[a] Tests 1 and 2 made with tomato leaves, test 3 made with apple leaves. The same crude antimycin suspension was used in all tests.

this reduction was probably due to an increase in leaf size; however, the increase in size of the apple leaves was negligible.

Effect of simulated rain. The effect of washing various preparations

TABLE 5.—*Effect of simulated rain on the control of apple scab by a crude antimycin suspension*

Potency of spray in units per ml.	Disease control[a]	
	Unwashed shoots	Washed shoots[b]
No.	Per cent	Per cent
4	81.7	59.2
2	77.5	66.2
1	36.6	25.4
0.5	2.9	0.0
0.25	0.0	0.0
1.125	0.0	0.0

[a] Each entry average of 8–10 shoots.
[b] Simulated rain, 2 inches.

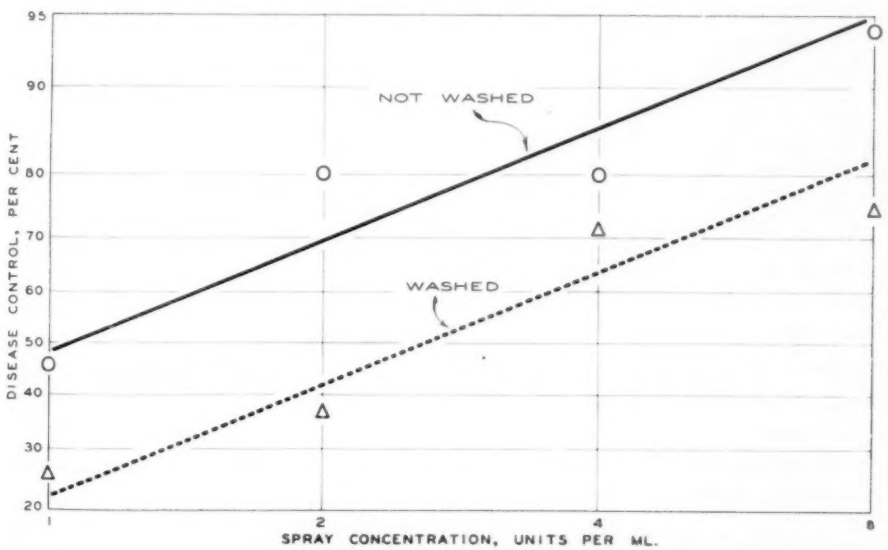

FIG. 4. Effect of simulated rain on the control of tomato early blight by one crude antimycin suspension. Single plant tests repeated in experiments completed at different times. Each point is the average of 3 tests.

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with simulated rain was studied with the aid of the foliage disease methods and the leaf disk assay. In two foliage disease tests, different suspensions

TABLE 6.—*Effect of simulated rain on the washing of different antimycin preparations from tomato leaves*

Preparation No.	Preparation type	Spray potency in units per ml.	Amount removed by 2 in. of rain
		No.	Per cent
165	Crude suspension	14	72.3 ± 6.6[a]
169	do	15	95
174	do	13	99
175	do	8	100
183	do	30	82, 87[b]
Do	do	60	94, 95[c]
B-8-18	[d]	12	93
B-8-43X	Crystalline antimycin A suspension	15	98, 100[b]

[a] Data from 6 experiments, 5 of which were completed at different times.
[b] Results from 2 experiments completed at different times.
[c] Results from 2 experiments completed at same time.
[d] Crude antimycin suspension partly purified (material derived from ether extract (8) suspended in water).

were indicated to be relatively resistant to washing (Table 5, Fig. 4). In another series of early blight tests with still another suspension, there appeared to be little or no washoff. On the basis of these tests it was re-

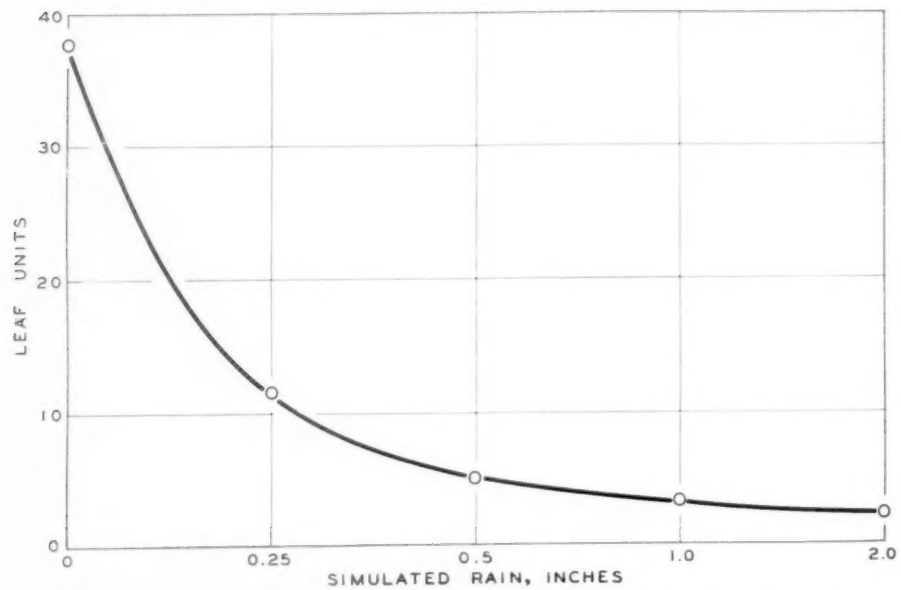

FIG. 5. Antibiotic activity remaining after tomato leaves were washed with various amounts of simulated rain.

ported that the crude material was resistant to washing (16). These results also suggested that some suspensions were more resistant to washing than others. Data from leaf disk assays substantiated this (Table 6).

With one suspension (No. 183, 60 units per ml.) most of the washoff occurred with the first ¼ in. of rain (Fig. 5). The washoff of the most resistant preparation was comparatively high (72.3 per cent). However, when the dosage-response curve is considered (Figs. 3, 4), such a reduction does not necessarily result in a corresponding reduction in percentage of disease control. It is concluded from these experiments that some antimycin preparations are comparatively resistant to washing and that others are not.

Several materials that have been used as stickers were tested in attempts to reduce washoff. Data obtained with leaf disk assays are given in table 7. No adjuvant appeared to have any marked effect in prevent-

TABLE 7.—*Effect of certain adjuvants on the removal of crude antimycin suspensions from tomato leaves by simulated rain*

Adjuvant	Amount of activity removed by 2 in. of rain	
	Prep. 165[a]	Prep. 183[b]
	Per cent	Per cent
PEPS[c]	81	76.9
Vulcanized rapeseed oil[d]	84	83.6
Soybean oil emulsion[e]	72	.....
None	71	82.0

[a] Average of 3 tests completed at different times.
[b] Results from 1 test.
[c] Polyethylene pentasulphide, 1 in 400 (21). Courtesy of B. F. Goodrich Chemical Co.
[d] 1 lb. per 100 gal. (12). Courtesy of the Stamford Rubber Supply Co.
[e] Soybean oil (0.25 per cent) emulsified with the aid of gum ghatti (0.005 per cent).

ing washoff. Under different drying conditions or with repeated applications, perhaps washoff would be lessened.

Effects of certain insecticides. The mixing of certain insecticides with crude preparations did not indicate loss of antibiotic activity. The insecticides tried were 50 per cent wettable DDT (DuPont), 2 lb. per 100 gal.; benzene hexachloride (Eastman, Pract.), 1 lb. per 100 gal.; hexaethyltetraphosphate (Planetary Chemical Co., Blot), 1 pint per 100 gal.; nicotine sulphate (Tobacco By-Products and Chem. Corp., Black Leaf 40), 2.5 pints per 100 gal., and lead arsenate, 2 lb. per 100 gal.

In one series of tests, the effects of these insecticides on the antibiotic material on the plant were investigated. A crude antimycin suspension was sprayed on a series of tomato plants (6–8 in. high) and the plants were dried; then sprays of the various insecticides were applied to separate plants. The two applications were made to avoid variations in deposit encountered when the antibiotic and insecticides were mixed before spraying. After 24 hr., leaf disk assays were made. Results indicated that as much antibiotic remained on leaves sprayed with various insecticides as on those that received no insecticide.

It is therefore concluded that under the conditions of these tests the insecticides had little if any effect on antibiotic activity. The effects of the

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antibiotic preparations on the insecticidal properties of the various compounds are not known.

Slide-germination tests. In tests with *Glomerella cingulata*, the LD 50 for one crude suspension (8.1 units per mg.) was found to be 1.07 μg. per cm.^{2} and that for standard Bordeaux (2) was 0.68 μg. per cm.^{2} (Fig. 6). On the basis of purity, expressed as units per mg., the predicted LD 50 for crystalline antimycin A was estimated to be *ca.* 0.008 μg. per cm.^{2} In actual tests, however, the LD 50 was more than 0.4 μg. per cm.^{2}, the highest concentration that was available. This is an additional evidence

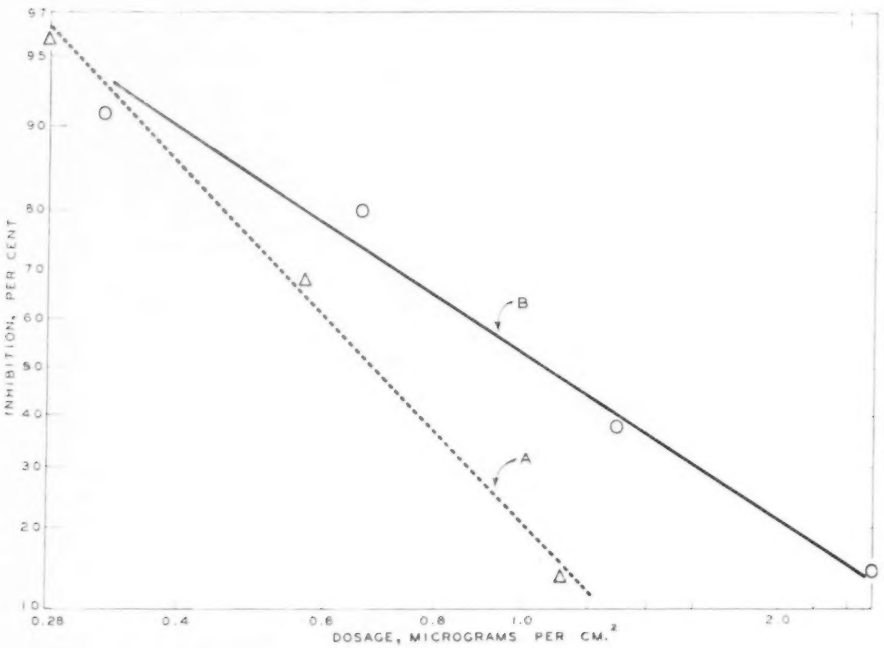

FIG. 6. Dosage-response curves for standard Bordeaux mixture (A) and one crude antimycin suspension (B) as determined by slide-germination tests using *Glomerella cingulata*.

(8) that inhibiting substances other than antimycin A may be found in the crude preparations.

Phytotoxicity. No phytotoxic effects have been noted with aqueous sprays of 100 units per ml. of the crude or crystalline suspensions on young tomato, bean (*Phaseolus vulgaris* L.), cucumber (*Cucumis sativus* L.), or cowpea plants under greenhouse conditions. Less potent crude suspensions have been sprayed on apple and pea (*Pisum sativum* L.) plants with no injury. No visible residues have ever been noticed.

The toxicity to man or experimental animals is not known, although studies on this subject are under way. No toxic effects have been observed by any of the people concerned with the various phases of the work.

DISCUSSION

In the present study it was demonstrated that antibiotic preparations derived from cultures of an unidentified *Streptomyces* species were effective as protectant fungicides in the greenhouse. Similar preparations from different fermentations of this organism, however, differed markedly in a number of properties and in disease controlling potentialities.

The effective preparations varied in texture, purity, amount retained on the leaf, resistance to washing with simulated rain, etc. The amount of activity (units per ml.) required for disease control also appeared to differ with various preparations (Tables 1, 2, 3, 5, and Figs. 3, 4), although these variations possibly may have been associated with differences in deposit or with experimental conditions that could not be controlled with the available facilities. Whether these variations in the crude preparations are caused by impurities, by antimycin A, or possibly by additional antibiotics is not known. However, the capacity to withstand washing did not appear to be associated with antimycin A, since this material was washed readily from the leaves.

It is concluded from these investigations that certain preparations of antimycin have possibilities as protectant fungicides. Small amounts were effective and were compatible with insecticides. The material was comparatively resistant to washing, and there were no visible residues. Phytotoxicity did not appear to be important. On the other hand, loss of activity on the leaf has been noted, and little is known about the toxicity of the material to man. The problem of differences in various preparations appears to be one of antibiotic production and control—a problem that seems solvable and one that is not peculiar to the fermentation industries.

The investigation thus far has been more concerned with the potentialities of antibiotic materials for combating plant diseases than with the possible practical adaptations of antimycin as a protectant fungicide. The high antibiotic effect of the various preparations on many fungi and their efficiency in the control of apple scab and tomato early blight in the greenhouse give encouragement to further studies of these preparations and of other antibiotics in relation to plant disease control.

SUMMARY

Various crude antibiotic preparations derived from cultures of an unidentified species of *Streptomyces* were effective as protectant fungicides in controlling apple scab and tomato early blight in the greenhouse. Preparations made from different fermentations varied in a number of properties, including the capacity to withstand washing from leaves. Certain crude preparations were comparatively resistant to washing, whereas crystalline antimycin A, derived from the crude material, was washed readily from the leaves. With the passage of time a loss in antibiotic activity on plant leaves was observed. No phytotoxicity was observed, and

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crude preparations appeared to be compatible with a number of insecticides.

The leaf disk assay for determining the amount of antibiotic on leaves is described.

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STUDIES ON THE MECHANISM OF RESISTANCE OF CRUCIFEROUS PLANTS TO PERONOSPORA PARASITICA[1]

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(Accepted for publication March 11, 1949)

An understanding of the mechanism of resistance is of prime importance in the breeding of plants resistant to disease. A comparative study was made of the host-parasite relationships and the environmental effects on the development of downy mildew in susceptible and resistant varieties of cruciferous plants, so as to throw light on the nature of their resistance to the fungus *Peronospora parasitica* (Pers.) de Bary.

MATERIALS AND METHODS

Most of the experimental work was done in Chengtu, China, from 1943 to 1944, and part of it was repeated in Nanking in 1947. Cruciferous varieties with different degrees of susceptibility or resistance were chosen: namely, Chinese rape (*Brassica Chinensis* L.), cabbage (*B. oleracea* L.), and radish (*Raphanus sativus* L.). The inoculum used consisted of the sporangia of *Peronospora parasitica brassicae* race 2 and *Peronospora parasitica raphani* (5). Leaves from which inoculum was to be taken were thoroughly washed in water so as to remove sporangia that had formed and also any surface contaminants that might be present. They were then placed in a moist chamber to secure a new crop of sporangia for inoculum. The spore suspension for inoculation was made by brushing sporangia into sterile tap water with the aid of a clean brush. Spore suspensions were atomized on the surfaces of detached leaves or of seedlings, and the inoculated leaves were kept in a moisture-saturated atmosphere for 1 day or longer. Light was admitted into the moist chamber through the glass cover. The inoculated plants, after remaining in the moist chamber for 1 or more days, were taken out and kept in the greenhouse while the disease developed.

PENETRATION BY THE PATHOGEN

Tsu (4) reported that the germ-tubes of sporangia of *Peronospora parasitica* penetrate the epidermis of the host directly and invade the interior through intercellular spaces; but he does not mention the method of infection on resistant varieties. An effort was made in the present experiment to find out whether the germ-tube penetrates resistant and immune plants.

[1] Paper No. 66 from Department of Entomology and Plant Pathology, University of Nanking, Nanking, China.

[2] The author wishes to acknowledge his great indebtedness to Dr. C. T. Wei for many valuable directions and criticisms, and to Dr. L. Ling and Dr. K. H. Lin for reading the manuscript.

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Chinese rape, cabbage, and radish were chosen for such a test. Chinese rape is susceptible to race 2 of *Peronospora parasitica brassicae*, cabbage is resistant, and radish is immune. Chinese rape is immune from *P. parasitica raphani*, cabbage is resistant, and radish is susceptible to it. Cross-inoculations were made on detached leaves with *Peronospora parasitica brassicae* race 2 and *P. parasitica raphani*. One day after inoculation, pieces of epidermis were stripped from the inoculated leaves with a forceps, stained with cotton blue, and observed under a microscope.

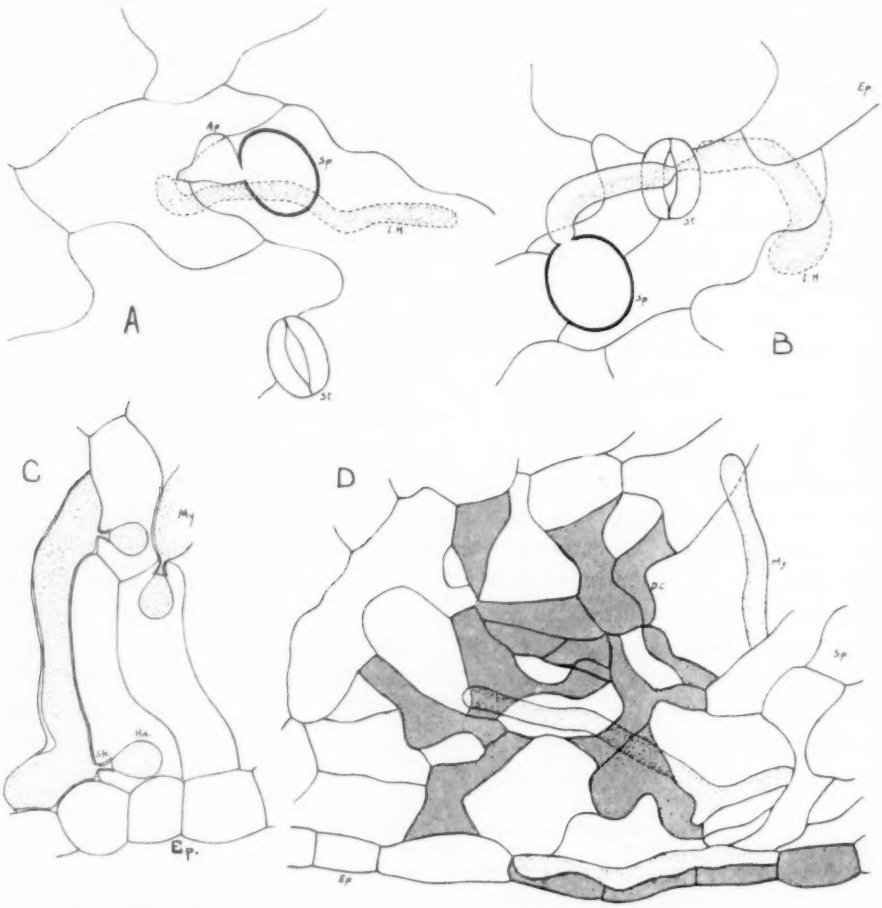

FIG. 1. *Peronospora parasitica brassicae* race 2. Above, entry of germ tube of the sporangium, through (A) an epidermal cell and (B) a stoma. Below, mycelium in tissue of (C) the susceptible Chinese rape host, and (D) the immune radish host. Legend: Sp, sporangium; Ap, appressorium; IH, infection hypha; My, mycelium; Ha, haustorium; Sh, sheath; Ep, epidermis; St, stoma; Sp, spongy mesophyll tissue; DC, dead host cells.

Both direct penetration and stomatal entry of the germ-tube were clearly observed on susceptible hosts, on the resistant hosts, and even on immune ones. In the course of direct penetration, the tip of the germ-tube swelled to form an appressorium. The infection hypha growing from the appressorium was greatly constricted as it penetrated the epidermal

cell with which it came in contact (Fig. 1, A). In the case of stomatal entry, the germ-tube of the sporangium also was constricted during the passage through a stoma (Fig. 1, B). Direct penetration was more frequently observed than was entry through a stoma. On the resistant and immune varieties, both types of penetration occurred. Later development of the infection hypha was greatly restricted on such hosts.

DEVELOPMENT OF THE PATHOGEN IN HOST TISSUE

There is a great diversity in the development of the pathogen after its entry into the plants, depending upon the degrees of their susceptibility. In the susceptible host, the invaded parts became discolored after 5 to 6 days, and the lesions gradually increased in size. On the resistant variety, only small necrotic spots, rather restricted in size, appeared on infected parts. Growth of the mycelium was greatly suppressed, an observation verified by study of stained sections of host tissue.

Healthy, detached leaves of Chinese rape and radish nourished in 5 per cent glucose solution were inoculated with sporangia of *Peronospora parasitica brassicae* race 2 and *P. parasitica raphani*, respectively. One set of Chinese rape and radish leaves was provided as a control in which no inoculation was made. Inoculated portions of leaves were fixed in Karpechenko's solution 2, 4, and 6 days after inoculation; the paraffin method was used for making sections, which were then stained with safranin and light green or haemotoxylin. Direct observation of leaves cleared with chloral hydrate and stained with cotton blue was sometimes used.

A series of observations revealed significant differences in the development of the infection hypha after the invasion of susceptible and immune hosts. After the infection hypha of *Peronospora parasitica brassicae* race 2 penetrated the susceptible rape host, the mycelium spread in all directions without limitation through the intercellular spaces of the leaf mesophyll, and at the same time sent globular or pyriform haustoria into the host cells. The base of each haustorium was usually surrounded by a sheath or membrane apparently of host origin (Fig. 1, C). On the other hand, in the immune radish variety the host cells surrounding the infection locus of *P. parasitica brassicae* race 2 became necrotic within 4 days after inoculation. A short hypha spread from the point of ingress without forming haustoria; in some cases hyphae apparently pushed against the host cells but failed to penetrate. Surrounding the invading mycelium were the dark brown, deformed, and dead host cells (Fig. 1, D). Similar phenomena were observed in the host tissues of radish and rape when inoculated with *P. parasitica raphani*. Apparently the resistance of cruciferous plants to *P. parasitica* is due to the death of the host cells, which prevents the establishment of the pathogen in the host tissue.

SPORULATION

An attempt was made to determine whether there were any differences in fungus sporulation on the lesions of susceptible and resistant hosts.

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Cross-inoculations with sporangia of *Peronospora parasitica brassicae* race 2 and *P. parasitica raphani* were made separately on Chinese rape, cabbage, and radish grown in pots and also on their detached leaves placed in Petri dishes with moistened paper at the bottom.

Race 2 of *Peronospora parasitica brassicae* sporulated abundantly on infected seedlings of Chinese rape exposed for 1 day to a saturated atmosphere. Necrotic spots without fungus sporulation appeared on cabbage, and there was no visible sign of infection on radish seedlings. The *P. parasitica raphani* sporulated abundantly on radish seedlings but produced only necrotic spots without spores on cabbage seedlings. There was no sign of infection on rape seedlings.

Results were entirely different on detached leaves. Neither fungus sporulated on detached leaves of the susceptible hosts. On the hosts that were usually immune from attack, each fungus developed sufficiently to cause necrotic spots.

A similar experiment was made with detached leaves nourished in a 5 per cent glucose solution, Knop's solution, or tap water and placed near a window to receive light. Again necrotic spots appeared on the hosts that were usually immune. The fungi sporulated, however, on the susceptible hosts.

EFFECTS OF ENVIRONMENTAL FACTORS ON THE DEVELOPMENT OF THE DISEASE

An attempt was made to determine the environmental factors that might cause a change in the expression of symptoms of the host. In the inoculation experiments, particularly with detached leaves of immune varieties, black necrotic spots appeared under conditions of weak light and prolonged moisture. Consequently, two factors, light and moisture, were tested separately for their effects on the host reactions.

Effect of Light

The downy mildew caused by an obligate parasite was particularly sensitive to light. Light probably affects the host first and then in turn affects the development of the pathogen. Schaffnit (3), Forward (1), and Hart and Zaleski (2) have proved that the expression of rust in a susceptible host is more or less altered under weak light or in darkness.

Seedlings of Chinese rape and radish were inoculated with *Peronospora parasitica brassicae* race 2 and *P. parasitica raphani*. Twelve pots of each host were used for each fungus and were held for 1 day in the inoculation chamber. Then two pots of Chinese rape and two of radish were placed in normal sunlight in the greenhouse, while all other pots were removed to a room with weak light. Each day, sets of two pots of Chinese rape and radish seedlings were transferred from the weak light to normal sunlight.

Conspicuous necrotic spots appeared on the immune hosts in the weak light (Table 1), and such spots became more obvious and also more nu-

TABLE 1.—*Effect of weak light on the infection of Chinese rape and radish seedlings by Peronospora*

Inoculum and number of days in weak light	Infection on Chinese rape		Infection on radish	
	Percentage	Type[a]	Percentage	Type[a]
P. parasitica brassicae race 2				
0	100	S	0	I
1	100	S	21	NI
2	99	S	19	NI
3	100	S	46	R
4	100	S	95	R
5	100	S	100	R
P. parasitica raphani				
0	0	I	89	S
1	0	I	74	S
2	26	R	85	S
3	83	R	94	S
4	86	R	93	S

[a] I indicates immunity and no signs of infection; NI, near immunity and a few small necrotic spots; R, resistance and conspicuous necrotic spots; S, susceptibility and sporulation of the fungus.

merous as the time in weak light increased. On susceptible hosts each fungus developed and sporulated well regardless of the light conditions. The incubation period on all hosts generally was increased by 1 to 2 days when the illumination was weak.

Effect of Moisture

Sets of Chinese rape and radish seedlings were kept at a high atmospheric moisture so that their cells would be very turgid. They were inoculated with the two pathogens and kept in the moist chambers for varying times. The effect of light could not be completely eliminated because light within the moist chamber was always weaker than the normal sunlight of the greenhouse.

A high percentage of infection was always secured on the susceptible hosts and the fungus developed and sporulated normally regardless of whether the period of high moisture following inoculation was 1 day or 5 days. The incubation period usually was shortened by 1 or 2 days when moisture was high. A single day at high atmospheric moisture did not alter the immune reaction of radish seedlings for *Peronospora parasitica brassicae* race 2; but 2, 3, 4, or 5 days of high moisture following inoculation permitted the development of 3 to 11 per cent infection and the appearance of a few small necrotic lesions which changed the reaction to one of near immunity. With 5 days of high atmospheric moisture following inoculation, 2 per cent infection of *P. parasitica raphani* developed on rape seedlings and the reaction was a near immune reaction. On the whole, moisture probably was a less important factor than light in changing the host reaction to these pathogens.

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DISCUSSION

The mechanism of resistance was studied by comparative observations of the pathogen's entry, its mycelial and haustorial development, and its sporulation in susceptible and resistant or immune hosts. None of the hosts hinder entry of the pathogen because the germ tubes of the fungus either penetrate the epidermis directly or enter the stomata without difficulty. After entry, however, there is great diversity in development of the fungus. In a susceptible variety the mycelium spreads freely in all directions through the intercellular spaces of the leaf mesophyll, and numerous haustoria enter the host cells to nourish the pathogen. In a resistant or an immune variety, the host cells surrounding the invading hyphae apparently become intoxicated and die. Consequently the fungus encounters difficulties in forming haustoria, and it may die from starvation or possibly because of toxins from the dead host cells. The only real difference between resistance and immunity lies in the quantity of host cells killed. In an immune host, so few cells may be killed that no external signs of infection are evident; in a near-immune host, the dead host cells are sufficiently numerous so that a few small necrotic lesions appear; and in a resistant host even more of the host cells are killed and numerous necrotic spots occur on the infected plant. The pathogen sporulates rather easily in susceptible hosts, but not in resistant or immune hosts.

The abundance of sporangia in lesions on a susceptible host implies that the fungus obtains a large amount of nutrient from the host. Yarwood (6) reported that downy mildew infection and sporulation of the fungus might cause a 55 per cent decrease in fresh weight of onion leaves, a similar decrease of 48 per cent in spinach leaves, and a decrease of 17 per cent in hop leaves. Sporulation during a single night might result in a decrease of 5 per cent in the dry weight of infected leaves (6). No such depletions of host materials are to be expected in resistant or immune varieties on which the fungus does not sporulate.

Environmental factors also play a role in the development of downy mildew. Light seems to be a more important factor than atmospheric moisture because a weak light modifies the immune reactions of a host. Weak light allows a somewhat greater development of fungus mycelium with the result that numerous and conspicuous necrotic lesions may appear on a host that generally has very few or no external symptoms of disease in normal sunlight. Nevertheless, the variability in disease reaction of the resistant hosts never was sufficient to allow the pathogen to sporulate, and the resistance is considered a true inherent resistance which plant breeders may depend upon for producing disease-resistant varieties.

SUMMARY

Host-parasite relationships were observed by means of inoculation studies and histological material for *Peronospora parasitica brassicae* race 2 and *P. parasitica raphani* on three hosts, Chinese rape, cabbage, and

radish. Chinese rape is susceptible to the first fungus and immune from the second, while a reciprocal relationship exists for radish. The cabbage host is resistant to both fungi.

The pathogens enter all plants whether susceptible, resistant, or immune, by penetrating directly through the epidermal cells or by entering the stomata.

After penetration, the fungus mycelium easily grows through the intercellular spaces of the leaf mesophyll and sends haustoria into the host cells of a susceptible host. In the resistant and immune hosts, development of mycelium and formation of haustoria are curtailed and the surrounding host cells die.

The fungi sporulate abundantly on susceptible hosts, but necrotic spots characterize the infection on resistant hosts. No symptoms or only a few small necrotic spots appear on the immune hosts.

Weak light may alter the immune reaction of a host so that it changes to a near immune or a resistant reaction. The differences are due primarily to the number of host cells killed by the invading fungus.

High atmospheric moisture may alter slightly the immune reaction of a host, but moisture is a less important environmental factor than light.

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GENETICS OF CERATOSTOMELLA. I. STRAINS IN CERATOSTOMELLA FIMBRIATA (ELL. & HALS.) ELLIOTT FROM SWEET POTATOES[1]

E. O. Olson

(Accepted for publication March 5, 1949)

INTRODUCTION

The fungus *Ceratostomella fimbriata* (Ell. & Hals.) Elliott is the cause of the important field and storage disease of sweet potatoes known as black rot. The fungus is also of interest from a genetic standpoint since it belongs in a genus in which species have been reported as homothallic, heterothallic, or containing both homothallic and heterothallic strains.

While *Ceratostomella fimbriata* has been considered a homothallic species, self-sterile or nonperithecial cultures have been observed. In Louisiana such cultures were frequently obtained by L. H. Person while making routine isolations from black-rot-affected sweet potatoes. This indicated that there were different strains of this fungus.

The present studies were made to determine the occurrence of strains in *Ceratostomella fimbriata* and to find out how these strains differ. A brief report of part of this work has been made (21).

LITERATURE REVIEW

Previous studies of the genetics of various species of *Ceratostomella* have dealt primarily with the inheritance of the factors governing the perithecial stage. Certain species have been reported as heterothallic, *C. coerulea* Munch (20), *C. multiannulata* Hedge. & Davidson (9), *C. paradoxa* (de Seynes) Dade (8), *C. pluriannulata* Hedge. (14), *C. radicicola* Bliss (5), and *C. ulmi* Buisman (6, 23), and others, including *C. adiposum* Sartoris (22) and *C. quercus* Georg. (20), as homothallic. The species *C. fagi* Loos may consist of both homothallic and heterothallic strains. Loos (18) observed that single ascospore cultures produced perithecia but Buisman (7) found that single conidium cultures fell into two self-sterile groups and it was necessary to pair members of these two groups to obtain perithecia. Leach (16) observed that single ascospore cultures of *C. ips* Rumb. produced both perithecial and nonperithecial strains. When conidia were isolated from the perithecial strain, cultures of both strains were obtained. Conidia from the nonperithecial strain produced only the nonperithecial strain and cultures of the nonperithecial strain did not form perithecia when paired.

Ophiostoma multiannulatum (Hedge. & Davidson) Melin & Nannf., synonymous with *Ceratostomella multiannulata*, has been the subject of physiological and genetic studies by Fries (12) and Fries and Trolle (13). They reported that three nonallelic genes control the reduction of sulfates.

[1] These studies were a result of support by Joseph E. Seagram and Sons, Inc., Louisville, Ky.

C. ulmi seems to consist of a number of strains which differ in cultural characters and pathogenicity (24, 25).

Various studies on the cytology of *Ceratostomella fimbriata* have been reported. Elliott (11) inferred that this species had heterothallic tendencies, while Mittman (20), Andrus and Harter (2, 3), and Gwynne-Vaughan and Broadhead (15) considered the fungus homothallic. Andrus and Harter (3), however, did isolate nonperithecial cultures. Barnett and

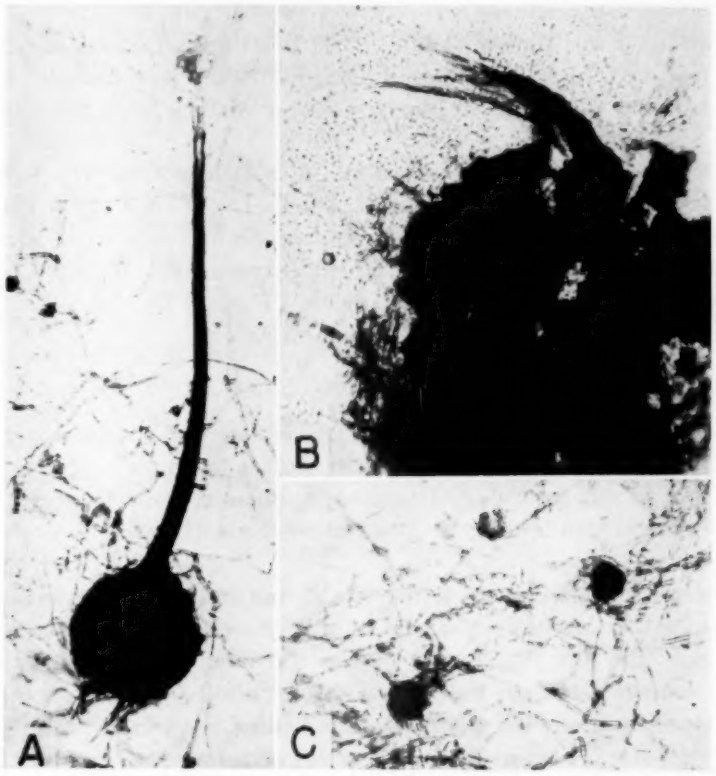

FIG. 1. A. Perithecium of self-fertile 1 strain of *Ceratostomella fimbriata* with typical long neck. B. Perithecium of self-fertile 4 strain with short neck. C. Sclerotium-like bodies in self-sterile 1 strain.

Lilly (4) reported that thiamin was necessary for perithecium production by *C. fimbriata*.

DESCRIPTION OF STRAINS

In the course of the present studies, strains were isolated which were self-fertile like the original parent, but which differed in other characters. Also, some strains were isolated which produced no perithecia when grown alone, and these likewise differed in certain other characters. For convenience, the ten strains reported in this paper are divided into two groups, self-fertile and self-sterile. The descriptions of each of the ten strains, based on growth on potato-dextrose agar, are given below.

Self-Fertile Strains

Self-fertile 1 was the wild type strain, which usually was isolated from black-rot lesions on sweet potatoes. It produced perithecia with long necks (Fig. 1, A) and abundant endoconidia.

Self-fertile 2 was similar to self-fertile 1 except that it grew more slowly, and the mycelium in culture had a bluish cast. Perithecia were beaked but tended to clump together. It was obtained as a variant from a cross between the strains self-fertile 4 × self-sterile 1.

Self-fertile 3 was similar to self-fertile 1 but grew faster and produced more aerial mycelium. Endoconidium production was perhaps slightly less than in the wild type. It occurred as a variant from the cross self-fertile 4 × self-sterile 2.

Self-fertile 4 appeared as a variant in isolations from the perithecia formed by pairing self-fertile 1 and self-sterile 1. It grew faster than self-fertile 1 in the early stages of growth and produced more aerial mycelium. Endoconidia were abundant. The salient characteristic of this strain was the production of short-necked perithecia (Fig. 1, B), which were fimbriate, like those of the other self-fertile strains.

Self-Sterile Strains

Self-sterile 1 produced sclerotium-like bodies, probably protoperithecia (Fig. 1, C), in culture, and an abundance of endoconidia. It was culturally similar to self-fertile 1. It was first isolated from black-rot lesions on sweet potatoes and occurred constantly among the ascospore progenies of self-fertile 1.

Self-sterile 2, similar to self-sterile 1 in that sclerotium-like bodies and abundant endoconidia were produced, was characterized by a slower growth rate and the bluish cast of the cultures. It was obtained constantly from the ascospore cultures of self-fertile 2.

Self-sterile 3 produced sclerotium-like bodies, grew faster than the two previous self-sterile strains, had more aerial mycelium, and produced fewer endoconidia. It was obtained from the ascospore progeny of self-fertile 3.

Self-sterile 4 produced no sclerotium-like bodies and formed few endoconidia. It was consistently found among the ascospore cultures of self-fertile 4.

Self-sterile 5 produced no sclerotium-like bodies, but formed abundant endoconidia, and grew more slowly than self-sterile 1. It was found as a single variant in the ascospore progeny of a self-fertile 1 isolate.

Self-sterile 6, similar to self-sterile 5, also produced no sclerotium-like bodies. It was somewhat darker and produced few endoconidia. It occurred as a recombination type from the cross, self-sterile 5 × self-sterile 1.

MATERIALS AND METHODS

The cultures of *Ceratostomella fimbriata* on which the present investigations were based were isolated from black-rot lesions on sweet potatoes or

were obtained from cooperators in other Southern states. The medium ordinarily used for culturing this fungus and for comparing cultural types was potato-dextrose agar.[2] Special media described later were used in a few experiments. Single spores, isolated with a micro-manipulator, were germinated on hanging drops of agar suspended over glass rings. Very few single spores germinated on freshly made agar drops. It was, however, found that when the hanging drops of agar were kept for three days over a water-saturated filter paper before single spores were isolated, the germination was raised from approximately 10 to 50–90 per cent. After the single spores germinated, the hanging drops were transferred to agar slants in test tubes. After 2 weeks the cultures were classified as to cultural type. Two methods were used to combine or to cross nonperithecial isolates with isolates of other cultural types. One method was to allow isolates to grow together. The second method was a "spermatizing" technique, where spores from the test isolate were added to week-old cultures of nonperithecial isolates. The second method made crosses possible between self-sterile isolates and self-fertile isolates. Certain self-sterile isolates "spermatized" in this manner formed long-necked perithecia within 3 days in contrast to the 7 or more days required for perithecium formation when self-fertile isolates were grown alone.

ASCOSPORE ISOLATIONS FROM SELF-FERTILE STRAINS

When single ascospores were isolated, it was observed that nonperithecial (self-sterile) as well as perithecial (self-fertile) cultures were se-

TABLE 1.—*Results of single ascospore isolations from 4 self-fertile strains of Ceratostomella fimbriata*

Parental strain	No. cultures tested[a]	Number of isolates of each strain in ascospore progenies		
		Cultural strains	No. cultures	Total
Self-fertile 1	103	Self-fertile 1 (Parent)	942	
		Self-sterile 1	634	
		Self-sterile 5	1	1577
Self-fertile 2	6	Self-fertile 2 (Parent)	268	
		Self-sterile 2	151	419
Self-fertile 3	2	Self-fertile 3 (Parent)	23	
		Self-sterile 3	23	46
Self-fertile 4	11	Self-fertile 4 (Parent)	42	
		Self-sterile 4	43	85
TOTAL				2127

[a] Two strains were obtained from every culture.

[2] Formula of potato-dextrose agar used to make one liter:
Water extract from 200 gm. of peeled potatoes
10 gm. dextrose
20 gm. agar-agar
Water to make 1000 cc.
Sterilize one hour at 15 lb. pressure

cured. To determine the ratio in which the two strains occurred, ascospores were isolated from 103 cultures of the self-fertile 1 strain and from a lesser number of three other self-fertile strains. The results are given in table 1.

From each culture of the four self-fertile strains tested, self-sterile strains were obtained in the ascospore progeny. A 3:2 ratio of parental strains to the variant strains was obtained in ascospore isolations from the self-fertile 1 strain.

To determine whether or not both self-fertile and self-sterile strains occurred in the same perithecium, ascospores were isolated from individual perithecia. The 22 individual perithecia studied included 11 of the self-fertile 1 strain, 2 of the self-fertile 2 strain, and 9 of the self-fertile 4 strain. From each of these 22 perithecia the self-fertile parental strain and its self-sterile variant strain were isolated.

A number of self-fertile 1 cultures were obtained from black-rot lesions on sweet potatoes from 7 states other than Louisiana, and isolations from single ascospores were made. The results of these isolations, given in

TABLE 2.—*Occurrence of self-sterile strains in ascospore progenies of self-fertile strains of Ceratostomella fimbriata from 8 states*

Source of isolate	Number of single ascospore isolates of each cultural type		
	Self-fertile 1	Self-sterile 1	Total
Alabama	15	8	23
Georgia	6	1	7
Louisiana	190	137	327
Mississippi	6	1	7
North Carolina	111	55	166
Tennessee	17	14	31
Virginia	10	6	16
West Virginia	53	33	86
TOTALS	408	255	663
Per cent	61.5	38.5	100.0

TABLE 3.—*Strains isolated from cultures of the self-fertile 1 strain in successive generations*

Generation	No. cultures tested	Self-fertile 1 (parent)	Self-sterile 1 (variant)	Total
First	1	12	4	16
Second	1	8	5	13
Third	1	4	1	5
Fourth	1	3	2	5
First	1	31	17	48
Second	16	107	79	186
Third	1	38	30	68
First	1	56	17	73
Second	1	41	19	60
TOTALS	24	300	174	474

table 2, show that both the self-fertile 1 and self-sterile 1 strains were obtained in the ascospore progenies from every culture.

The occurrence of cultures of the self-sterile 1 strain in the ascospore progenies of self-fertile 1 strains continued for four ascospore generations (Table 3).

CONIDIAL ISOLATIONS

The large number of cultures of self-sterile strains appearing among the single ascospore cultures of self-fertile strains made it seem advisable to determine what strains might appear among the cultures from endoconidia. Isolations of single endoconidia were made from 7 of the strains.

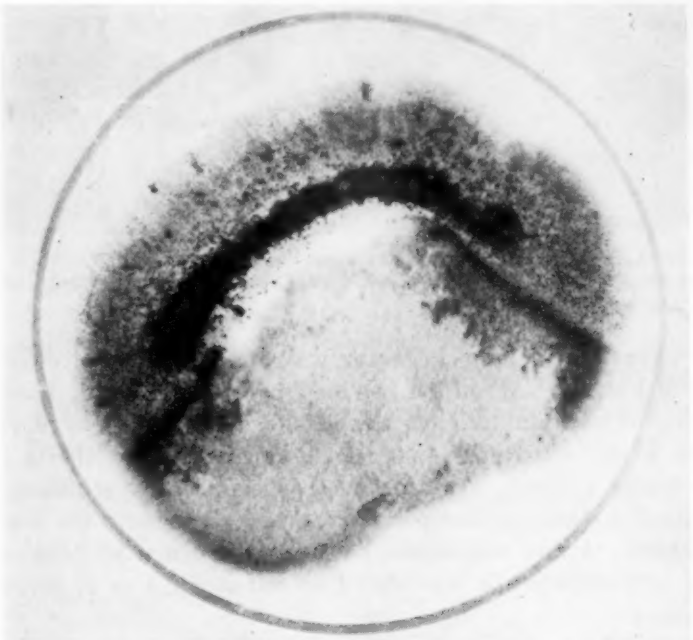

FIG. 2. Perithecia at line of contact between self-fertile 1 and self-sterile 1 strains of *Ceratostomella fimbriata.*

Of the 581 conidial cultures isolated and compared, all were of the respective parental strain. These included 293 from the self-fertile strains 1, 2, and 4, and 288 from the self-sterile strains 1, 2, 4, and 5.

When endoconidia were stained they were found to be uninucleate. This indicated that the mycelium of these strains was not heterocaryotic in the manner reported by Dodge (10) in *Neurospora tetrasperma.*

COMPATIBILITY OF CULTURES

When cultures of the two original strains (self-fertile 1 and self-sterile 1) isolated from black-rot lesions were paired, a line of perithecia formed where they grew together (Fig. 2). A line of perithecia also formed where isolates of self-fertile 4 and self-sterile 1 grew together.

When a water suspension of endoconidia from the self-fertile 4 strain was placed on the mycelium of the self-sterile 1 strain, fertile long-necked perithecia appeared at that spot within 3 days (Fig. 3). No perithecia

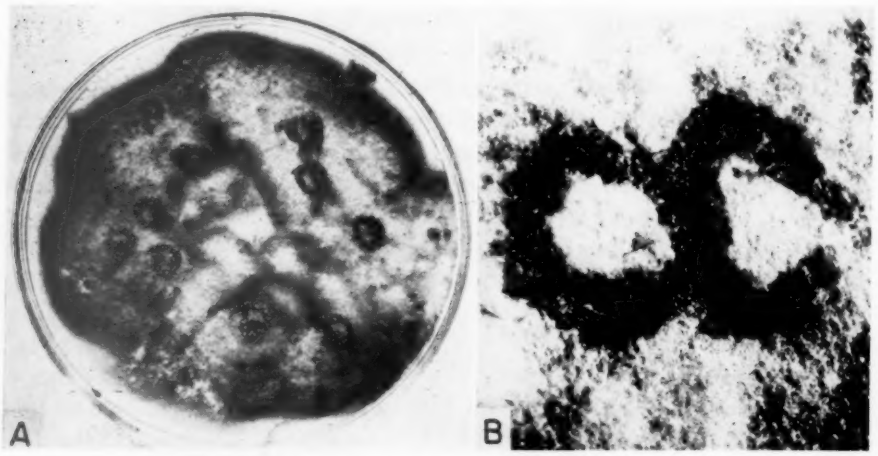

FIG. 3. A. Perithecia formed where spore suspension of self-fertile 4 strain was placed on mycelium of self-sterile 1 strain of *Ceratostomella fimbriata*. B. Enlarged view showing long-necked perithecia.

formed where only a drop of water was placed on the mycelium of the self-sterile 1 strain. When the reverse cross was made, *i.e.*, when a water suspension of endoconidia from the self-sterile 1 strain was placed on the mycelium of the self-fertile 4 strain, no long-necked perithecia appeared; the self-fertile 4 strain, which produced short-necked perithecia, thus did not produce the long-necked perithecia which formed on the line between the self-fertile 4 and self-sterile 1 strains. When a suspension of self-fertile 1 or 2 endoconidia was placed on self-sterile 1 or 2, perithecia also were formed.

These results indicated that there are sex or compatibility groups in this species, and that the self-sterile 1 or 2 strains functioned as a female, forming long-necked perithecia in combination with self-fertile isolates.

The ability of 8 of the 10 strains to form perithecia in various combinations was studied. Such combinations were made either by growing two strains in the same Petri plate or by "spermatizing" one strain by the other. Combinations which were not tested are indicated by a question mark in table 4, where the results are summarized.

The self-sterile strains 1 and 2 produced long-necked perithecia in combination with self-fertile strains 1, 2, and 4. Self-sterile 1 and self-sterile 5, as well as self-sterile 2 and self-sterile 5, produced long-necked perithecia in combination. When endoconidia from one strain were placed on the mycelium of the other strain, self-sterile strains 1 and 2 again functioned as females, and self-sterile 5 functioned as a male, since perithecia would form on self-sterile 1 and 2, but not on self-sterile 5.

TABLE 4.—*The results of combining cultures of 8 strains of Ceratostomella fimbriata*

Strain	Self-fert. 1	Self-fert. 2	Self-fert. 4	Self-ster. 1	Self-ster. 2	Self-ster. 4	Self-ster. 5	Self-ster. 6
Self-fertile 1	−	−	−	+	+	−	−	−
Self-fertile 2	−	−	−	+	+	−	−	?
Self-fertile 4	−	?	−	+	+	−	−	−
Self-sterile 1	+	+	+	−	−	−	+	−
Self-sterile 2	+	+	+	−	−	−	+	−
Self-sterile 4	−	−	−	−	−	−	−	−
Self-sterile 5	−	?	−	+	+	−	−	−
Self-sterile 6	−	−	−	−	−	−	−	−

+ = perithecia formed. − = no perithecia formed. ? = combination not tried.

INHERITANCE OF CHARACTERS AFFECTING SEX AND CULTURAL CHARACTERISTICS

The occurrence of perithecia where certain of the strains were paired offered an opportunity to determine whether or not fertilization occurred, and also to study the inheritance of some of the genetic factors determining the differences among the various strains.

Table 5 gives the results of ascospore isolations from 5 pairings between self-fertile and self-sterile strains. The genotypes of the various strains on the basis of the results are given. In the first pairing, between self-fertile 1 and its variant self-sterile 1, the ascospore progeny included 307 of the self-fertile parent, 297 of the self-sterile parent, and 37 cultures of self-fertile 4 strain. These 37 cultures were probably mutants, since the back-cross, self-fertile 4 × self-sterile 1, gave four strains in approximately

TABLE 5.—*Results of ascospore isolations from 5 compatible combinations between self-fertile and self-sterile strains of Ceratostomella fimbriata*

Parental strains	Parental genotypes	Progeny strains and genotypes							
		SF 1 ABC	SF 2 ABc	SF 4 A_1BC	SS 1 AbC	SS 2 Abc	SS 4 A_1bC	Other strain	Total
Self-fertile 1 × Self-sterile 1	ABC × AbC	307		37[a]	297				641
Self-fertile 2 × Self-sterile 1	ABc × AbC	36[b]	28		32	9[c]		23[d]	128
Self-fertile 2 × Self-sterile 2	ABc × Abc		7			6			13
Self-fertile 1 × Self-sterile 2	ABC × Abc	20[b]	16		16	5[c]			57
Self-fertile 4 × Self-sterile 1	A_1BC × AbC	176	1[a]	195	158		155		685
							TOTAL		1524

[a] These are considered variants which are not recombinations from the cross of the parental isolates.

[b] It seemed that this group could be divided into two minor sub-groups, but sufficient studies were not made to be positive of separating them for genetic studies.

[c] It is not known why so few of this group occurred.

[d] Insufficient information to classify.

a 1:1:1:1 ratio in 685 cultures, as well as one culture of another strain, self-fertile 2. The isolations from the first pairing, self-fertile 1 × self-sterile 1, would not prove that fertilization between the two strains took place, since ascospores from self-fertile 1 cultures growing separately also gave these strains in a 3:2 ratio. The crosses self-fertile 2 × self-sterile 1 and self-fertile 1 × self-sterile 2 indicated that a fertilization had taken place in these combinations since four strains occurred in the progeny of each cross. It is therefore presumed that fertilization also took place in the cross self-fertile 1 × self-sterile 1.

On the basis of these results, it is considered that these self-sterile variants differ from their self-fertile parents by one factor at locus B, and that self-fertile strains 1 and 4 have different genes at locus A. Self-fertile 2 strain differed from self-fertile 1 at a third locus, C.

Ascospore isolations also were made from the perithecia formed where certain self-sterile strains came together. The results are given in table 6,

TABLE 6.—*Results of isolations of crosses between self-sterile strains of Ceratostomella fimbriata*

Parental strains	Parental genotypes	SF 1 ABC	SS 1 AbC	SS 5 aBC	SS 6 abC	SF 2 ABc	SS 2 Abc	Total
Self-sterile 1 × Self-sterile 5	AbC × aBC	113	101	116	84			414
Self-sterile 2 × Self-sterile 5	Abc × aBC	3	3	6		9	7	28
					TOTAL			442

together with the postulated genotypes. From one cross, self-sterile 1 × self-sterile 5, 414 cultures were isolated and classified. Four strains occurred in the progeny in approximately a 1:1:1:1 ratio. These strains included the two parental strains, the self-fertile 1 strain from which the two parental strains were obtained, and the recombination strain expected where the parents differed by two factors. This strain, self-sterile 6, as shown in table 4, was not compatible with the other strains. Self-sterile 2 also was paired with self-sterile 5. Only 28 cultures were isolated from this cross, but 5 of the 8 strains expected were obtained, and, of these, 12 were self-fertile isolates.

These results indicate that there are at least two loci controlling the production of perithecia in this fungus. The pairing of two self-sterile strains, each containing only one of the two genes necessary for perithecium production, results in the formation of fertile perithecia. The recombination of the genes necessary for perithecium production reconstitutes the homothallic or self-fertile strain.

EFFECT OF THIAMIN ON PERITHECIUM PRODUCTION

Barnett and Lilly (4) demonstrated that thiamin was necessary for the production of perithecia by *Ceratostomella fimbriata*. The isolates

which they studied would be the same as the self-fertile 1 strain reported in this paper. In the present study, isolates of various strains were grown on Czapek's and on potato-dextrose agar, with and without the addition of thiamin. Self-fertile 1 or self-fertile 4 isolates did not produce perithecia unless 5 μg. of thiamin was added to the media. However, the addition of 100 μg. of thiamin to Czapek's media or to potato-dextrose agar did not induce the production of long-necked perithecia by self-fertile 4 isolates, which produced short-necked perithecia on potato-dextrose agar. Additions of 100 μg. of thiamin to the same two media did not induce the production of perithecia by isolates of the self-sterile 1, self-sterile 3, or self-sterile 5 strains. The other cultural types were not tested.

Thus, while the appearance of some of these strains can be simulated by thiamin-deficiency, these strains, when grown on potato-dextrose agar, apparently represent distinct genetic entities rather than thiamin-starved isolates.

PATHOGENICITY OF STRAINS

The pathogenicity of 9 strains was determined by inoculation tests. The inoculations were made by inserting conidia or mycelium into wounds in the surface of sweet potatoes. After inoculation, the sweet potatoes were placed in paper sacks and examined for lesions after 7 or more days had passed. The comparisons of pathogenicity were made on the basis of the area of the black-rot lesions and the depth of penetration.

One hundred and seventy-three cultures representing 9 strains were tested for pathogenicity. Self-fertile strains 1, 2, 3, and 4 produced typical lesions, although those caused by self-fertile 2 were smaller. Self-sterile strains 1, 2, 3, and 4 were also pathogenic, although self-sterile 2, like its parental strain self-fertile 2, also produced small lesions. The strain self-sterile 5 was nonpathogenic.

Forty-six cultures from the cross self-sterile 1 (pathogenic) × self-sterile 5 (nonpathogenic), were tested for pathogenicity to sweet potatoes. These 46 cultures included the 4 strains which were obtained from this cross. The results (table 7) show that pathogenicity and nonpathogenicity were inherited in a 1:1 ratio (Fig. 4). This indicated that the self-sterile 5 strain lost its pathogenicity when it arose from the parental strain,

TABLE 7.—*Pathogenicity to sweet potato of ascospore progeny from the cross between the two strains, self-sterile 1 × self-sterile 5, of Ceratostomella fimbriata*

Cross	Cultural type of the progeny	Genotype	No. of isolates tested	Pathogenicity
Self-sterile 1 (AbC)	Self-fertile 1	ABC	11	Pathogenic
×	Self-sterile 1	AbC	13	Pathogenic
Self-sterile 5 (aBC)	Self-sterile 5	aBC	15	Nonpathogenic
	Self-sterile 6	abC	7	Nonpathogenic
	TOTAL		46	

self-fertile 1, and that the pathogenicity factor in question was apparently located at the A locus.

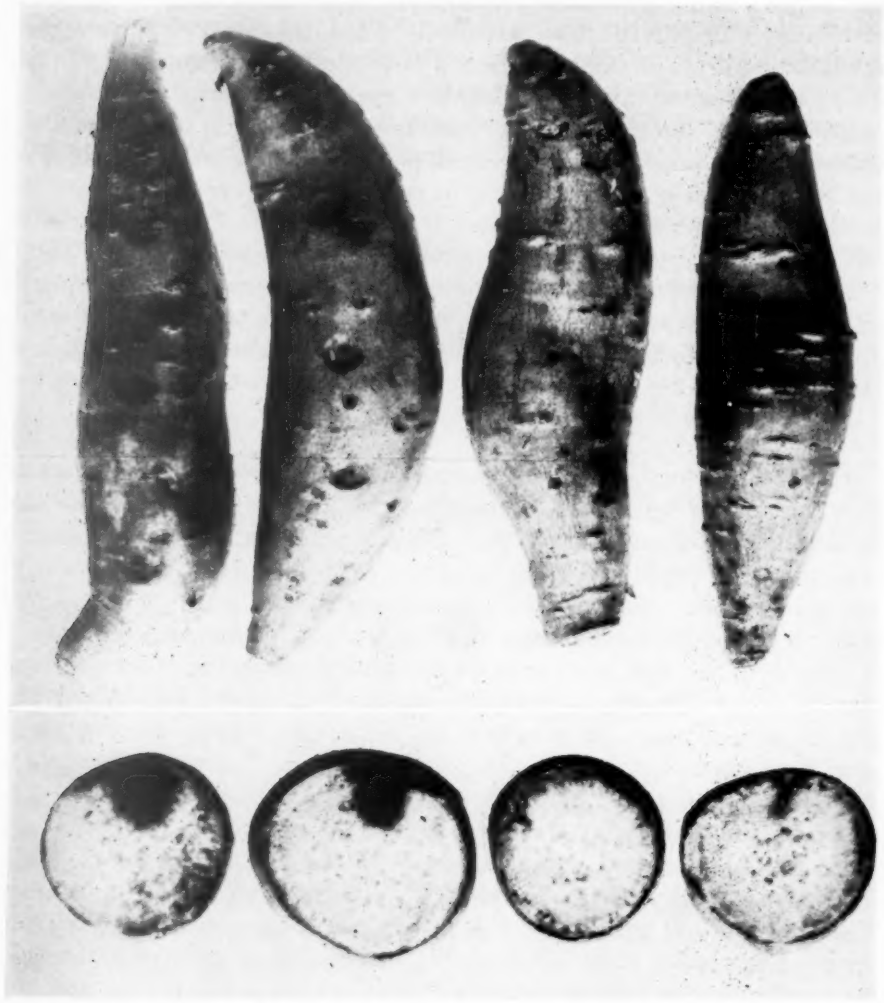

FIG. 4. Surface view and cross section of sweet potatoes inoculated with 4 strains of *Ceratostomella fimbriata* derived from a cross between the self-sterile 1 and self-sterile 5 strains. The two potatoes on left inoculated with pathogenic, the two on right with nonpathogenic strains.

DISCUSSION

The continued occurrence in *Ceratostomella fimbriata* of a large number of self-sterile strains in ascospore cultures of self-fertile strains is similar in many respects to the phenomenon reported by Lindegren (17) in *Neurospora* and in *Glomerella* by Andes (1) and Lucas, Chilton, and Edgerton (19). Other species of *Ceratostomella*, as *C. ips.* (16), also give self-sterile strains from ascospores.

Of the mechanisms which could be responsible for the results with the self-fertile strains, segregation and mutation appear to be the most likely ones. For segregation to occur, the fusion nucleus in the ascus must be heterozygous. Since ascospores are apparently uninucleate and the nuclei present in them are a result of meiosis, it would appear that the nuclei that fuse in the ascus in the next generation should be alike. It is possible that mutation would give genetically different nuclei which would fuse in the ascus and result in the segregation for parental self-fertile strains and self-sterile ones. If mutation is responsible, a selection of variant nuclei probably occurs in the formation of perithecia and ascospores, otherwise strains should have been found among the endoconidial isolates. If no mutation occurs, segregation implies that there are two chromosomes in the nucleus of the self-fertile strain which are capable of pairing at meiosis but which carry different genes at the same locus. The fusion nucleus in the ascus would then carry more than two chromosomes which were homologous. This would necessitate the adoption of the concept that the haploid stage of the fungus was 2n and the diploid 4n. The ratios from the crosses made among the various strains were those expected from the crosses of haploid rather than diploid isolates. It would also be necessary to account for the high occurrence of the self-sterile 1 strain and very low occurrence of the self-sterile 5 strain from the self-fertile 1 strain.

The nature of the differences between homothallic and heterothallic fungi is an interesting problem. In *Ceratostomella fimbriata* it would seem that strains are homothallic when they possess in the same nucleus two main factors which govern the initiation and formation of perithecia and ascospores. If one of these factors is absent the strain is self-sterile and if both are absent the strain becomes neuter. If the two factors are present in nuclei in different thalli the cultures behave as heterothallic ones and when brought together form perithecia and ascospores.

SUMMARY

1. Ten strains of *Ceratostomella fimbriata*, differing in pathogenicity, morphology, and compatibility were isolated and described. These strains were obtained from the ascospore progeny of cultures isolated from black-rot lesions on sweet potato or from the crosses of variants of the same origin.

2. The ascospore progeny of 4 self-fertile strains included not only the parental self-fertile strains but its respective self-sterile strain. Single conidium isolates from single-spore cultures were of the parental type.

3. Long-necked perithecia formed on the line between self-fertile and certain self-sterile strains, as well as between certain self-sterile strains. Isolates of still other strains did not produce perithecia alone or in combination with other self-sterile strains.

4. The ascospore progeny from perithecia formed by combinations of strains included the parental types alone, or both parental and recom-

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bination types. The results of these crosses suggest that a combination of at least two independently inherited genes are necessary for perithecium production.

5. Ascospore isolations from a homothallic strain gave rise to heterothallic strains. The progeny of the cross of two heterothallic strains included isolates apparently identical to the original homothallic strain.

6. Half of the progeny of the cross of pathogenic × nonpathogenic isolates were apparently nonpathogenic to sweet potato.

7. The addition of thiamin to Czapek's medium was necessary for perithecium formation by isolates capable of forming perithecia on potato-dextrose agar. The addition of thiamin to potato-dextrose agar did not induce the formation of perithecia by isolates which produced no perithecia upon potato-dextrose agar.

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REACTIONS OF THREE SPECIES OF DODDER TO HOST PLANTS USED IN VIRUS DISEASE INVESTIGATIONS

C. F. LACKEY

(Accepted for publication March 16, 1949)

Work by Bennett[1, 2] showed that some dodders were useful in the transmission of various viruses to different host plants. Following this work many investigators have used dodder in the transmission of viruses to a wide variety of host plants. Although many of the dodders have an extensive host range, their usefulness in virus transmission is limited by the extent of this range. Studies were undertaken on three species of dodder to determine how well they would parasitize certain varieties of host plants. A brief report of this work has been given[3].

MATERIALS AND METHODS

The two species of dodder which were used in Bennett's[1] first studies were used in these experiments. These are the relatively large-stemmed one, *Cuscuta subinclusa* Dur. and Hilg., and the very small-stemmed species, *C. californica* Choisy, both of which grow vigorously on healthy sugar beet petioles. The host plants used were those most commonly used in the virus work at this laboratory,—sugar beet, Turkish tobacco (*Nicotiana tabacum* L. var. Turkish), tree tobacco (*N. glauca* Graham), tomato, and two varieties of cucurbits, cucumber and Zucchini squash. *C. americana* L., a dodder with still larger stems than either of the other two, has recently been added to the list of dodders tested by Bennett[4]. It was, therefore, studied anatomically also by the writer on sugar beet, tomato, and tobacco.

The study of the reaction of the three species of dodder on different host plants was made from free-hand sections of fresh tissues and from material fixed in alcohol-formalin-acetic solution. The sections of the fixed material were cut 20 μ thick. These preparations were stained in dilute iron-haematoxylin without destaining, and counter-stained with dilute safranin. Sometimes light green in clove oil was used with these two stains or as a counter-stain with the safranin alone.

EXPERIMENTAL RESULTS

Sugar beet and tobacco are good hosts for all the dodder species studied. The dodders grow luxuriantly on these host plants without any noticeable effect on the tissues through which the haustoria pass. Figure 1, A shows

[1] Bennett, C. W. Acquisition and transmission of viruses by dodder (*Cuscuta subinclusa*). (Abstr.) Phytopath. **30**: 2. 1940.

[2] ———. Studies of dodder transmission of plant viruses. Phytopath. **34**: 905–932. 1944.

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[4] Bennett, C. W. Unpublished data.

Cuscuta subinclusa on a beet petiole. Haustorial hyphae precede the main body of the haustorium. The xylem vessels of this dodder develop and fuse with those of the host plant when the haustorium has reached the bundle, as in the blending of tissues in a successful graft. This is shown in figure

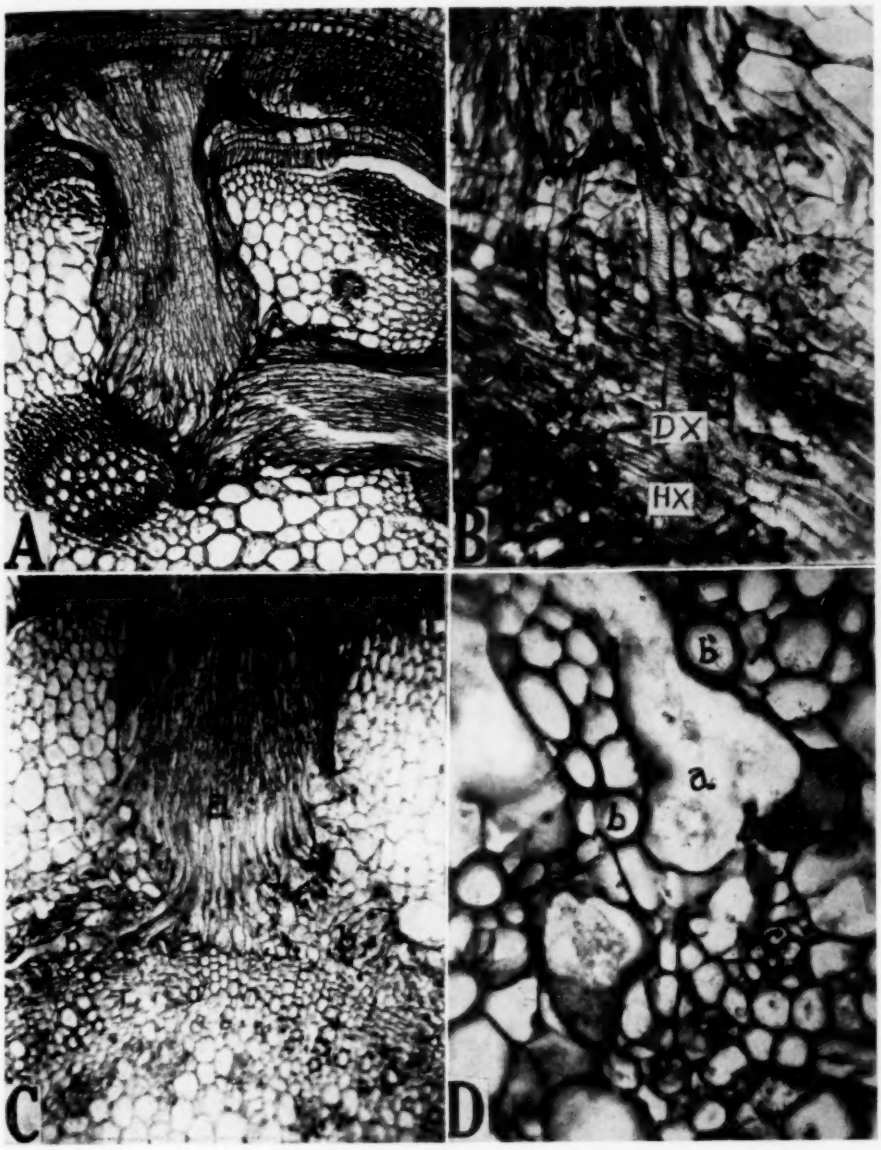

FIG. 1. Dodder haustoria in beet and tobacco. A. Two haustoria of *Cuscuta subinclusa* contacting vascular bundle of sugar-beet petiole. B. Longitudinal section of beet petiole showing how xylem vessels of dodder haustorium (dx) blend with xylem of host plant (hx). C. Same dodder on *Nicotiana tabacum* with haustorial hyphae (b) of haustorium (a) contacting external phloem. D. Haustorial hypha (a) which has penetrated beyond xylem (b) and is growing in direction of internal phloem of tobacco stem.

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1, B. The other dodders developed in the same way on the sugar beet.

On the stem of tobacco the growth of dodder is somewhat different. In tobacco the vascular bundles form a distinct ring between cortex and pith. There are groups of phloem cells forming an external and an internal phloem on each side of the ring. A dodder haustorium sends its hyphae along the outer edge of this vascular ring making contact with the external phloem tissue first as shown in figure 1, C. After this contact by the hyphae the xylem vessels usually develop and contact the xylem tissues of the host plant. The haustorial hyphae sometimes grow on through the ring of xylem vessels and xylem parenchyma toward the internal phloem as in figure 1, D.

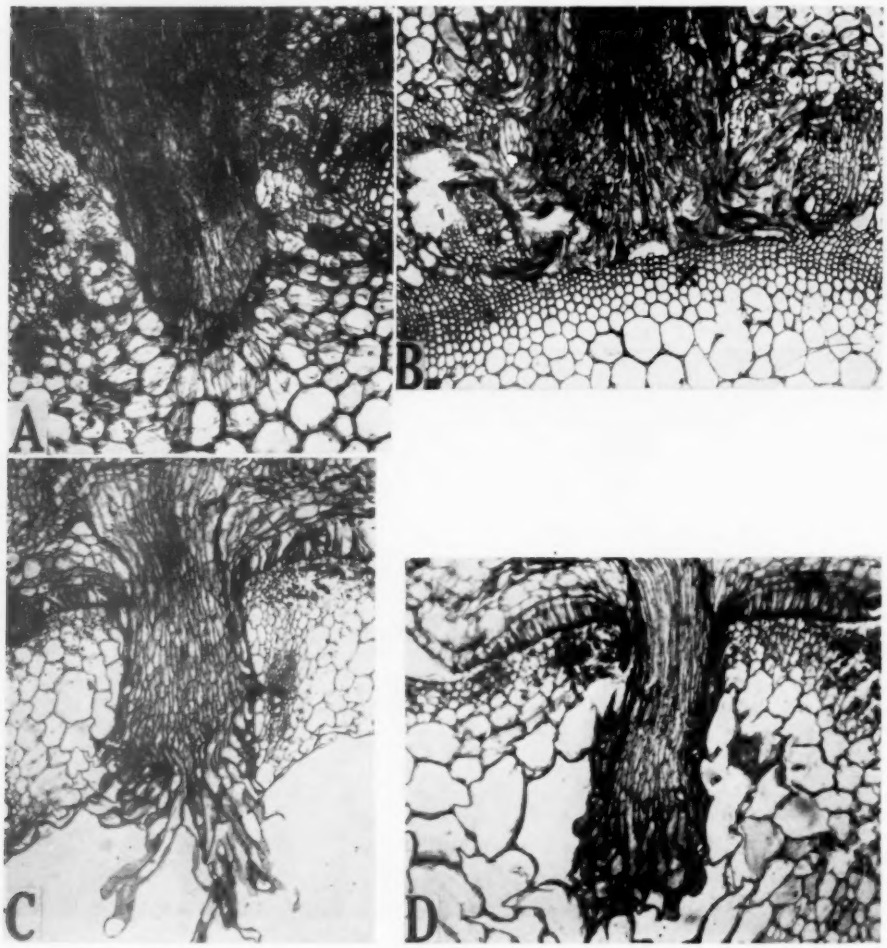

FIG. 2. Dodder on tobaccos and cucurbits. A. *Cuscuta americana* haustorium on *Nicotiana tabacum*, having penetrated beyond xylem ring and external phloem where hyphae make only limited contact with internal phloem. B. *C. americana* on *Nicotiana glauca*; haustorium has failed to pass through xylem ring (x), and hyphae (h) have made good contact with external phloem. C. *C. subinclusa*, having grown through stem of cucumber into hollow center making very little vascular contact. D. Haustorium of *C. subinclusa* stranded among large cells of Zucchini squash stem, making no vascular contacts.

Cuscuta americana grows more luxuriantly on tree tobacco than on Turkish tobacco. A study of the host tissues of these varieties of tobacco parasitized by *C. americana* suggests a possible explanation. It was observed that the haustorium and its hyphae in Turkish tobacco grew past the external phloem and contacted only a limited amount of internal phloem (Fig. 2, A), while on tree tobacco growth of the haustorium was confined to the bark where the greatest amount of phloem tissue exists (Fig. 2, B).

Two species of cucurbits were tested. The first was cucumber (variety Long Green) and the other, Zucchini squash. The cucumber has thin, hol-

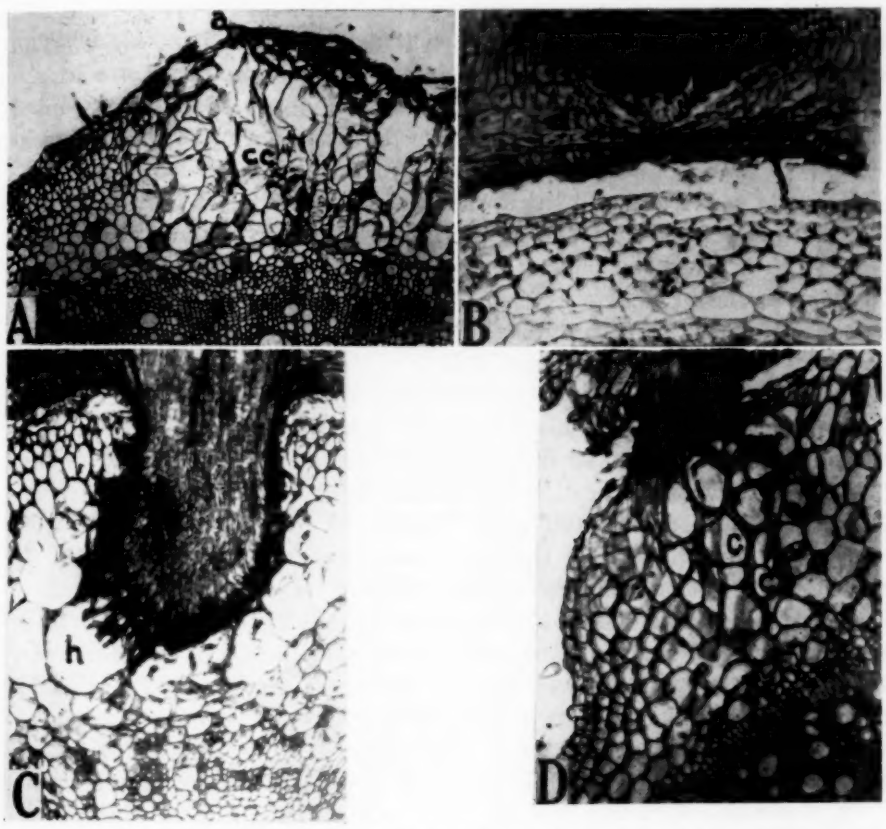

FIG. 3. A. Transverse section of tomato stem which has been lightly contacted (a) by dodder haustorium, producing severe hypertrophy and disorganization of cortex cells (cc); normal cells at (nc). B. Transverse section of tomato stem (t), and dodder stem (d) which has encircled tomato stem for 6 weeks while growing on tobacco plant; haustorial initials (hi) have failed to develop into haustoria in any part of the encircling dodder stem. C. *Cuscuta americana* on tomato stem; haustorium has caused some hypertrophy of cell (h) but not sufficient to prevent some vascular contact D. *C. californica* on tomato stem, making a vigorous growth with no hypertrophy or abnormal reaction of host cell and haustorial hyphae (c).

low stems and relatively small cells. *Cuscuta subinclusa* grew for a while on this species but not vigorously. In a majority of cases, as shown in figure 2, C, the haustoria grew right through the stem into the hollow center, with few vascular contacts. In other cases the haustoria grew di-

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rectly to the vascular bundles. Zucchini squash has a large stem with big cells and a large hollow center. *C. subinclusa* will not grow on this plant without being connected to a favorable host. A tobacco plant with a vigorous growth of *C. subinclusa* on it was placed among the stems of a Zucchini squash and the fast-growing stems of the dodder twined around the stems of the squash leaves. The dodder was allowed to grow for 4 or 5 weeks until it seemed well established on the squash stems. When the dodder was cut loose from the tobacco plant and left on the squash plant it began to decline rapidly. Microscopic examinations made of these squash stems with dodder on them showed (Fig. 2, D) that the haustorium had developed and had grown into the host stem as deeply as it does in other host stems. The squash cells surrounding the haustorium were abnormally large and apparently devoid of contents. The haustorium appeared to be stranded among them and unable to make contact with a vascular bundle for nourishment.

Cuscuta americana will live for a considerable time on the tomato but does not grow luxuriantly on it. It induces some hypertrophy of cortex cells (Fig. 3, C). The haustoria do not make as good contact with the vascular tissues of tomato as they do with tissues of beet petioles. Some haustorial hyphae apparently are isolated by the large cells.

Cuscuta californica will grow well for long periods on the stem of the tomato plant. This dodder also attaches itself to the petiole of a compound leaf where it grows much more luxuriantly than on the stem. The relationship between this small dodder and the tomato stem is similar to that between *C. subinclusa* and the tobacco stem or beet petiole. There apparently is no abnormal cell growth produced by the contact of dodder haustorium and the stem of the host plant, as is shown in figure 3, D. In this figure, the haustorial hyphae are unusually long. The usual growth of the haustoria of this dodder in the tomato stem is similar to its growth on beet and tobacco, with one exception. The haustoria do not penetrate very far into the tomato stem before long hyphae are sent out to the vascular tissue. The haustorium shown in figure 3, D has not penetrated much beyond the epidermal layer of cells. On beet or tobacco the haustoria penetrate the cortex much deeper before their hyphae reach the vascular ring.

The reaction between *Cuscuta subinclusa* and the tomato plant is the most unusual of all host reactions observed. A mutual antagonism seems to exist between the host and the parasite, resulting in an abnormal development of the haustorium as it comes in contact with the tomato stem. No penetration takes place, but this contact induces an extremely abnormal growth in the cortex parenchyma of the tomato. Figure 3, A, a transverse section of tomato stem so contacted, shows most cells extremely hypertrophied and disorganized, with a few normal cells at the left side. Figure 3, B shows the effect of mere contact between a haustorium and an old tomato stem. *C. subinclusa*, while growing vigorously on a tobacco plant, was trained on a mature tomato plant stem. The dodder completely en-

circled the tomato stem and grew for 6 weeks while still attached to the original host. When cut loose from the tobacco plant, the dodder declined rapidly and soon died. Sections were made of the tomato stem with the encircling dodder stem. On no part of the encircling dodder were haustoria produced. It is evident from figure 3, B that the contact stimulus started the development of haustorial initials, but they never developed beyond a rudimentary stage. The tomato stem being fully mature at the time the dodder was attached, no hypertrophy developed in the cortex of the stem. This indicates an inhibitory effect of the tomato stem on haustorial development, since this dodder produces fully developed haustoria even on contact with a wooden stick.

SUMMARY AND CONCLUSIONS

Studies were made on the reactions of three species of dodder (*Cuscuta subinclusa* Dur. and Hilg., *C. californica* Choisy, and *C. americana* L.) on the following host plants commonly used in virus disease research: sugar beet, tobacco, tomato, and two varieties of cucurbits (cucumber and Zucchini squash).

These three dodders grow well on sugar beet and tobacco. They do not grow vigorously or for very long on cucumber. *Cuscuta subinclusa* will establish itself on Zucchini squash but will not live independently on it. The haustoria fail to cross the barrier of large cells in the squash stem to contact vascular tissue.

Cuscuta americana will grow for a short period on tomato but not vigorously. The haustoria induce some cell hypertrophy in the tomato stem and that, to some degree, seems to prevent haustorial hyphae from contacting vascular tissue.

Cuscuta subinclusa will not establish itself on the tomato stem but mere contact of the haustoria with the stem may produce severe hypertrophy and abnormal development of the cortex cells. This dodder growing on a tobacco plant was allowed to encircle the main stem of a mature tomato plant. Sections made of the dodder on the tomato stem showed haustorial initials which never developed beyond the rudimentary stage. Since haustoria are developed upon contact even with a wooden stick, this indicates an inhibitory effect of the tomato stem on haustorial development.

Proper selection of the species of dodder and the host plant is necessary if one is to succeed in transmitting virus by dodder in any given case.

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INCIDENCE OF FUSARIUM INFECTION AS AFFECTED BY ROOT-KNOT NEMATODES

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(Accepted for publication March 18, 1949)

There are many statements in the literature to the effect that the severity of Fusarium wilt is increased by root-knot nematodes, although good supporting experimental evidence has been lacking. Indirect evidence has been obtained, however, indicating that when soil fumigants are used to control nematodes the severity of cotton wilt is reduced[2, 3]. Both *Heterodera marioni* (Cornu) Goodey and *Fusarium oxysporum* f. *gladioli* (Massey) Snyder and Hansen were encountered on gladiolus in a soil-fumigation experiment in the Rio Grande Valley of Texas, which raised the question concerning the effect of root-knot on the incidence of Fusarium infection. Consequently, experiments were set up at the Plant Industry Station, Beltsville, Maryland, to determine whether or not root-knot nematodes increase the incidence of Fusarium infection in gladiolus, in tomato, and in squash. The Fusaria used were vascular pathogens in gladiolus and in tomato, and a cortical pathogen in squash.

For these tests the plants were grown in steamed 8-in. clay pots filled with autoclaved soil consisting of a mixture of one part sand, one part muck, and one part compost. Four hundred pots were used in experiment 1, with 20 pots in each of 20 treatments. The different inoculation treatments are listed in table 1. In addition to those listed for Picardy gladiolus, there were odd-numbered companion treatments consisting of 20 pots of noninoculated Picardy gladiolus, 20 inoculated with population 15 of *Heterodera marioni*, and 20 inoculated with a mixture of populations 3B and 15 of *H. marioni* to serve as controls. Similar companion treatments were used with Dr. F. E. Bennett gladiolus, tomato, and squash in both experiments 1 and 2. Inocula were prepared as follows: the Fusaria were grown on sterile wheat, ground in a food chopper, and 2 qt. of this mixed with 30 qt. of soil; the nematodes were increased in the greenhouse on squash roots, which were then ground and mixed with soil. The *Fusarium oxysporum* f. *gladioli* used was a mixture of our isolates 106, 123, and 127 obtained from gladiolus corms; the *F. oxysporum* f. *lycopersici*

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[2] Smith, A. L. Control of cotton wilt and nematodes with a soil fumigant. Phytopath. 38: 943–947. 1948.

[3] Taylor, A. L., H. D. Barker, and P. H. Kime. Further observation on the nematode-Fusarium-wilt experiment at Lumberton, North Carolina. (Abstr.) Phytopath. 30: 710. 1940.

(Sacc.) Snyder and Hansen was F. L. Wellman's R5-6; and the *F. solani* f. *cucurbitae* Snyder and Hansen was obtained from W. C. Snyder. Two

TABLE 1.—*Effect of root-knot nematodes on the incidence of fusarium infection in gladiolus, tomato, and squash*

Treatment number[a]	Plants inoculated with	Relative root-knot index[b]	Number of plants infected with Fusarium[c]	Average number of days prior to harvest when symptoms appeared[d]
	Experiment 1			
	Gladiolus var. Picardy			
2	*Fusarium oxysporum* f. *gladioli*		60	19.0
4	*Fusarium oxysporum* f. *gladioli* plus *Heterodera marioni* 15		60	25.3
6	*F. oxysporum* f. *gladioli* plus *H. marioni* 15 and 3B		57	20.7
	Gladiolus var. Dr. F. E. Bennett			
8	*F. oxysporum* f. *gladioli*		56	44.6
10	*F. oxysporum* f. *gladioli* plus *H. marioni* 15		49	47.6
12	*F. oxysporum* f. *gladioli* plus *H. marioni* 15 and 3B		55	50.5
	Tomato var. Marglobe			
14	*F. oxysporum* f. *lycopersici*		14	18.3
16	*F. oxysporum* f. *lycopersici* plus *H. marioni* 3B	3.5	12	21.3
	Squash var. White Bush			
18	*F. solani* f. *cucurbitae*		20	37.7
20	*F. solani* f. *cucurbitae* plus *H. marioni* 3B	4.0	20	43.6
	Experiment 2			
	Squash var. White Bush			
22	*F. solani* f. *cucurbitae*		13	17.4
24	*F. solani* f. *cucurbitae* plus *H. marioni* 5	3.7	9	13.1
26	F. *solani* f. *cucurbitae* plus *H. marioni* 7	3.9	12	10.8
28	*F. solani* f. *cucurbitae* plus *H. marioni* 8	4.0	13	11.0
30	*F. solani* f. *cucurbitae* plus *H. marioni* 15	3.7	14	16.3

[a] The odd-numbered treatments are the controls and are not listed. See text.

[b] 0.0 indicates no galling and 4.0 indicates maximum galling. This index was obtained on companion plants inoculated with nematodes only.

[c] Sixty plants were inoculated in each of treatments 2 to 12, 20 in each of treatments 14 to 20, and 16 plants in each of treatments 22 to 30.

[d] This indicates the rapidity with which infection took place; *i.e.*, small numbers indicate that the incidence of infection was slower. The addition of root-knot nematodes did not result in a significant increase in the incidence of the disease except in treatments 4 and 12. These increases are significant at the 5 per cent level only.

populations of *H. marioni* were used, 3B and 15. Population 3B was obtained from potatoes from Harlingen, Texas, and population 15 was obtained from gladiolus from Linn, Texas. The soil in the pots was infested

as follows: 1 qt. of the autoclaved soil was placed in the bottom of each pot and this was covered with either 250 ml. of the Fusarium inoculum, 250 ml. of the nematode inoculum, or 250 ml. of each of the two inocula. The pots were then filled with soil. For the gladiolus experiments three No. 1 size corms, variety Picardy or Dr. F. E. Bennett, were planted per pot. For the squash experiments, 3 seeds of the variety Farr's Benning White Bush were planted per pot, and for the tomato experiments, 3 seeds of the Marglobe variety. The squash and the tomato plants were thinned to one per pot. The date of the appearance of the first symptoms of Fusarium infection in each plant was recorded, and this information analyzed statistically. All of the plants were harvested while the noninoculated ones were still green. At this time the gladiolus had finished flowering, and the squash and tomato had fruited heavily. The number of days to harvest after planting was 99, 93, and 129 days for gladiolus, squash, and tomato, respectively. The roots of the tomato and squash plants inoculated with nematodes alone were scored for galling and given a relative root-knot index.[4]

In a second experiment, additional populations of *Heterodera marioni* were used with *Fusarium* on squash since considerable variability in pathogenicity exists among races[5]. These populations were obtained as follows: population 5, from parsnip, grown near Falls Church, Virginia; population 7, from a Castella rubber tree propagated in a Florida nursery; population 8, from tomato plants grown in a greenhouse at Beltsville, Maryland; and population 15, from gladiolus. The soil was infested, the plants grown, and the data recorded in the same manner as in experiment 1. There were but 16 pots of squash per treatment in the second experiment, and these plants were all dug 110 days after planting.

The root-knot indices, numbers of plants infected with *Fusarium*, and the average numbers of days prior to harvest when symptoms appeared, are recorded in table 1, for both experiments. Severe galling occurred in the tomato and squash as is indicated by the high root-knot indices obtained from the plants inoculated with nematodes only. The average number of days prior to harvest when symptoms appeared seems to be a reliable criterion of disease incidence. Thus, higher numbers indicate an earlier, more rapid incidence of infection; and consequently if *Heterodera marioni* speeds up the incidence of Fusarium infection or if it causes it to be more severe, the figures for the *H. marioni-Fusarium* combinations should be higher than for *Fusarium* alone. The data indicate this except in the case of squash in experiment 2. However, with but two exceptions, none of the differences are statistically significant. Incidence of Fusarium infection was significantly greater at the 5 per cent level in gladiolus when

[4] Taylor, A. L. Field methods of testing for root-knot infestation. Phytopath. 37: 85–93. 1947.

[5] Christie, Jesse R., and Florence E. Albin. Host-parasite relationships of the root-knot nematode, *Heterodera marioni*. I. The question of races. Proc. Helminth. Soc. Washington 11: 31–37. 1944.

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combined with population 15 of *H. marioni* (treatment 4) and when combined with a mixture of populations 3B and 15 (treatment 12). These differences were not great, however, and it can be concluded that, under the conditions of these experiments at least, *H. marioni* had very little effect on the incidence of Fusarium infection. No conclusions can be drawn regarding the effect of different nematode populations.

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A WILT-INDUCING POLYSACCHARIDE FROM FUSARIUM SOLANI F. EUMARTII[1]

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(Accepted for publication March 21, 1949)

Among the several theories advanced in the last half-century to explain the wilting of plants affected with certain bacterial or fungus diseases, the toxin theory has been most generally accepted. A considerable amount of recent work has been concerned with culture filtrates of wilt organisms. It has been shown that these organisms, when grown upon synthetic media, produce toxic substances capable of wilting excised shoots of healthy plants. Although relatively few chemical analyses of culture filtrates have resulted in positive identification of the toxic substances, a number of different chemical compounds have been described as wilt-inducing substances.

The literature concerned with the wilt problem has been reviewed by Brown (1), Harris (7), and Gottlieb (6). Recent investigations have been made by Clauson-Kaas, Plattner, and Gäumann (2), Hodgson, Riker, and Peterson (9, 10, 11), Dimond (3), and Wolf and Wolf (13).

The purpose of this paper is to describe the properties and nature of a wilt-inducing substance present in culture filtrates of *Fusarium solani* f. *eumartii* (Carp.) Snyder and Hansen. This organism is the cause of a wilt and stem-end rot disease of potato.

EXPERIMENTAL WORK

Preparation of culture filtrates. A single spore culture of *Fusarium solani* f. *eumartii* was used in all experiments. The organism was grown on a modified Richard's solution of the following composition: KNO_3, 10 gm.; KH_2PO_4, 5 gm.; $MgSO_4$, 2.5 gm.; $FeCl_3$, 0.02 gm.; dextrose, 50 gm.; Haas and Reed's "A–Z" solution, 1 ml.; and distilled water, 1500 ml. The reaction of the nutrient solution was adjusted to pH 6.0 with K_2HPO_4 before sterilizing in an autoclave at 15 lb. pressure for 20 min. Roux flasks containing 200 ml. of the nutrient solution were inoculated with 3 loopfuls of conidia of the organism from cultures 10 days old. After incubation at room temperature for 14 to 20 days, the amber to purple culture solution, which was covered by a thick mycelial mat, was filtered through 3 layers of cheesecloth and then centrifuged at 5000 r.p.m. for 15 min. to remove most of the mycelial fragments and conidia. The pH of the culture filtrates was 8.0 to 8.5.

Wilting tests. Stems of healthy, rapidly growing Bonny Best tomato plants with 4 or 5 well-developed leaves were cut under water and then placed in test tubes containing 20 ml. of the solution to be tested.

[1] Excerpt from a thesis presented June, 1948, to the faculty of the Graduate School of Cornell University in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

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The first experiments were run in the laboratory and in the greenhouse. Rather erratic results were obtained in these experiments and it was thought that such factors as light, temperature, humidity, and air movement were not constant enough to give consistent results. Several wilt tests were then performed in a cold storage room where temperature was fairly constant at 13° C. in the dark. The plants were illuminated by four 500-watt General Electric CX (infrared, mild ultraviolet) lamps. The plants were placed on a table 3 ft. below the lights and were separated from the lamps by a glass plate. A 10-in. fan was used to create air movement in the chamber. The fan was directed toward the side of the chamber and the deflected air passed over the plants. At the beginning of each wilt test the lights were turned on and the fan was started. In a period of 10 hr., the duration of a wilt test, the temperature in the chamber gradually rose from 13° C. to 32° C. and the relative humidity decreased. The lights and fan were turned off and the temperature was allowed to return to 13° C. before another test was run. Consistent results were obtained from tests performed in the cold-storage room under the described conditions.

A minimum of 3 cuttings was used to test individual solutions in most experiments. Unless otherwise stated each experiment was repeated at least 5 times. In each of the wilt tests checks consisted of plants in sterile nutrient solution. In no case did the checks wilt.

The results of several preliminary tests with unadulterated culture filtrates indicated that a wilt-inducing substance was present. Usually within 1 hr. the cuttings were partially wilted. There was some loss of turgidity in the petioles and the upper part of the stems causing the leaves and top part of the cuttings to droop. By the time the tests were concluded the stems were flaccid and the cuttings had collapsed. At this state a cutting was considered to be completely wilted although the leaflet blades were still somewhat turgid.

The following study of the properties of the wilt-inducing substance was made primarily to determine the type of substance involved.

Dialysis. One hundred ml. of culture filtrate were placed in a cellophane dialyzing tubing (Will Corp. No. 6598). A small marble was placed within the membrane for the purpose of stirring the solution. Dialysis was carried out in a Kunitz-Simms (12) rocking-table dialyzer. Distilled water was passed through the glass tube surrounding the membrane at the rate of 1 gal. per hour. The tubing was removed from the dialyzer after 24 hr. and the solution was tested for wilt-inducing activity. The pH of the culture filtrates was 8.0 to 8.5 before dialysis and 6.5 to 7.0 after dialysis. A total of forty-seven 100-ml. samples were tested after dialysis. In these tests a total of 184 tomato cuttings was used. Checks in each test included nondialyzed culture filtrate, sterile nutrient solution, and distilled water. Tomato cuttings in the dialyzed culture filtrate showed partial to complete wilt in 2 to 10 hr. Plants in the non-

dialyzed culture filtrate wilted somewhat more quickly and more severely. Later experiments showed that when mineral salts were added to the dialyzed culture filtrate the wilt-inducing activity was increased. No wilting occurred in shoots placed in the sterile nutrient solution or distilled water. No tests were run on the dialysate. Nondialyzed culture filtrates gave a strong test for reducing sugars with Fehling's solution but dialyzed filtrates gave a negative test, indicating that dialysis was effective in removing substances of small molecular weight. These results showed that the toxic substance had a relatively large molecular weight.

Filtration. Since filtration through cheesecloth and centrifugation does not completely remove all conidia and mycelial fragments it was thought advisable to determine whether or not the culture filtrate could be filtered through a bacterial filter without removing the wilt-inducing substance. Dialyzed and nondialyzed culture filtrates were filtered through Seitz, Berkefeld, and fritted-glass bacterial filters. Whatman No. 2 filter paper also was included in the tests. Tomato plants showed little or no wilt in filtrates passed through any of the bacterial filters. The toxic substance did pass through the filter paper. Culture filtrates were tested for filterability at pH 2.5 and 9.0. The pH was adjusted with either 0.1 N hydrochloric acid or sodium hydroxide. Filtrates were readjusted to pH 7.0 and tested for wilt-inducing activity. The toxic substance did not pass through the bacterial filters at either pH 2.5 or 9.0. Adjusting the pH of unfiltered solutions to either 2.5 or 9.0 did not inactivate the toxic substance. It is assumed that the molecules of the substance are either too large to pass such filters or that they are adsorbed on the filter.

Precipitation with ethyl alcohol. Nondialyzed culture filtrates were treated with 2 volumes of 95 per cent ethyl alcohol. The precipitate that formed was dried over calcium chloride and then redissolved in distilled water. Wilt tests showed that the toxic substance had been precipitated by the alcohol. Dialyzed culture filtrates were treated with 2 and 5 volumes of 95 per cent ethyl alcohol. The solutions turned slightly turbid but no precipitate resulted that could be collected by filtration or centrifugation. An attempt was then made to determine whether or not the toxic substance could be precipitated from the dialyzed culture filtrate by alcohol if the solution were more concentrated. Tubing containing a dialyzed culture filtrate was removed from the dialyzer and placed in front of an electric fan and the solution was evaporated to dryness. The residue from dialyzed filtrates was active and could be kept in the dried state for several weeks without any apparent loss in activity. The residue from 100 ml. of dialyzed culture filtrate was redissolved in 20 ml. of distilled water. Five ml. of this solution were run into 25 ml. of cold (0° C.) 95 per cent ethyl alcohol at the rate of one drop per second with vigorous stirring. A gummy, gray precipitate resulted which was removed by centrifugation and dried. The precipitate obtained from 100 ml. of dialyzed culture filtrate was redissolved in 100 ml. of distilled water and then tested

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for wilt-inducing activity. The tests showed that the toxic substance was precipitated with the alcohol.

Solubility tests. The toxic substance appeared to be very soluble in water. Attempts were made to extract the toxic substance from dialyzed culture filtrates with organic solvents. Dried alcoholic precipitates from 50 ml. of dialyzed filtrate were shaken with 20 ml. of each of the following: ether, benzene, ethyl acetate, chloroform, and dioxan. The mixtures were allowed to stand for 6 hr. and then were filtered through paper. The filtrates were evaporated to dryness. An extremely small amount of residue was obtained from the filtrates. The residues collected on the filter papers and those obtained from the filtrates were redissolved in 25 ml. of distilled water and tested for wilt-inducing activity. In all cases the toxic substance appeared in the fraction not soluble in the organic solvent.

Effect of heat on nondialyzed culture filtrates. Since the wilt-inducing substance present in culture filtrates of *Fusarium solani* f. *eumartii* was reported by Goss (5) to be inactivated by boiling, experiments were performed to determine whether or not filtrates of the isolate used in these studies behaved similarly. One hundred ml. of nondialyzed culture filtrate (pH 8.0 – 8.5) were divided into two equal parts, one of which was placed in a steamer at 100° C. for 15 min. A precipitate formed on heating and this was removed by centrifugation. The heated and unheated solutions were adjusted to pH 7.0 with 0.1 N hydrochloric acid and the solutions were then tested for wilt-inducing activity. The results of such tests, involving 58 different cultures, showed that heating at 100° C. for 15 min. either partially or completely inactivated the wilt-inducing substance.

Effect of heat on dialyzed culture filtrates. The alcohol-insoluble precipitate from 100 ml. of dialyzed culture filtrate was dissolved in 80 ml. of distilled water. Heating this solution in the steamer for 15 min. to 2 hr. did not result in inactivation of the toxic substance. No precipitate formed on heating.

It was thought that the precipitate that resulted from heating nondialyzed culture filtrates consisted largely of phosphates or other substances present in the nutrient solution that are insoluble in hot, alkaline solutions. To test this hypothesis, the alcohol-insoluble precipitate from 100 ml. of dialyzed culture filtrate was dissolved in 80 ml. of sterile, nutrient solution. This was divided into two equal parts, one of which was adjusted to pH 5.9 and the other to pH 7.5. Each of these two solutions was divided into two equal parts, one of which was heated at 100° C. for 15 min. in a steamer and the other left unheated. A precipitate formed in the solution heated at pH 7.5. This precipitate was removed by centrifugation. No precipitate formed on heating the solution at pH 5.9. Wilt tests showed that inactivation occurred only in the solution heated at pH 7.5. In subsequent experiments the precipitate that formed on heating at pH 7.5 was redissolved by adjusting the solution to pH 6.0 and allowing

it to stand at room temperature for 24 to 48 hr. Wilt tests showed that the toxic substance was present in this solution. It thus appeared that heating the toxic substance in an alkaline Richard's solution resulted in removal of the substance along with the precipitate or that the substance was insoluble in the presence of nutrients under these conditions. Heating in the absence of nutrients at pH 7.5 had no effect on the wilt-inducing activity.

Chemical nature of the wilt-inducing substance. Several qualitative chemical tests were run on the alcohol-insoluble precipitate obtained from dialyzed culture filtrates. The procedures followed in performing the various tests were those given by Hawk, Oser, and Summerson (8). The furfural tests were performed according to the procedure given by Fearon (4). A summary of the reactions is presented in table 1. A negative test

TABLE 1.—*Reactions exhibited by the alcohol-insoluble precipitate from dialyzed culture filtrates of Fusarium solani f. eumartii*

Test	Reaction[a]	Test	Reaction[a]
Ninhydrin	–	Fehling's solution, before hydrolysis[b]	–
Biuret	–		
Millon's	–	Fehling's solution, after hydrolysis[b]	+
Xanthoproteic	+?	Nitro-chromic, after hydrolysis[b]	+
Trichloroacetic acid	–		
		Furfural	
Phosphomolybdic acid	–	Thymol, 15% HCl	–
		Thymol, 35% HCl	+
Molisch	+	Orcinol, 15% HCl	–
		Orcinol, 35% HCl	+
Osazone[c] after hydrolysis[b]	+	Iodine	–
Precipitation with C_2H_5OH	+		

[a] + positive reaction; — negative reaction; +? weak or incomplete reaction.
[b] 0.5 N HCl at 100° C. for 2 hr.
[c] Glucosazone type crystal formed.

for protein was obtained in the ninhydrin, biuret, and Millon's tests. The xanthoproteic reaction was weak and could not be interpreted as definitely positive or negative. No precipitation occurred with trichloroacetic acid or phosphomolybdic acid. Iodine gave no color reaction. The Molisch test for carbohydrate was positive. Aqueous solutions of the precipitate gave a negative test for reducing sugar with Fehling's solution. Hydrolysis was carried out by dissolving the alcohol-insoluble precipitate from 100 ml. of dialyzed filtrate in 0.5 N hydrochloric acid and heating for 2 hr. at 100° C. Solutions of hydrolyzed material gave a strong test for reducing sugar with Fehling's solution. The nitro-chromic reaction was positive after hydrolysis. In the furfural tests thymol gave no reaction in 15 min. at 100° C. with 15 per cent hydrochloric acid but gave a carmine color with concentrated hydrochloric acid at the same time and temperature. Orcinol reacted similarly. The furfural reactions indicated that the sugar

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was an aldohexose. Glucosazone crystals were formed in the phenylhydrazine reaction with hydrolyzed material. The phenylhydrazine test was negative with unhydrolyzed material.

Tests were performed to determine whether or not hydrolysis of the alcohol-insoluble precipitate inactivated the wilt-inducing substance. The alcohol-insoluble precipitate from 100 ml. of dialyzed culture filtrate was dissolved in 10 ml. of distilled water. This solution was divided into two equal parts. Sufficient concentrated hydrochloric acid was added to one part so that the concentration was 0.5 N. Both solutions were heated at 100° C. for 2 hr., then cooled, and adjusted to pH 7.0. Thirty ml. of distilled water were added to each of the two solutions which then were tested for wilt-inducing activity. Complete inactivation of the wilt-inducing substance resulted from the acid hydrolysis. The solution heated in the absence of acid was fully active. The acid-hydrolyzed solution gave a strong test for reducing sugar with Fehling's solution and the test with the unhydrolyzed solution was negative.

Results of the preceding tests indicated that the wilt-inducing substance was possibly a polysaccharide of high molecular weight or a mixture of polysaccharides. A polysaccharide was always associated with fractions exhibiting wilt-inducing activity. The polysaccharide was partially precipitated with saturated ammonium sulphate. In all cases the most active fraction contained the most polysaccharide.

Amount of material present in culture filtrates. The amount of material obtained by alcoholic precipitation from dialyzed filtrates was determined. The precipitates obtained from three 100-ml. portions of dialyzed filtrates were dried in an oven at 90° C. for 24 hr., then cooled in a desiccator at room temperature, and weighed. The weights of the precipitates were 46.2, 45.8, and 44.1 milligrams.

Nature of action of the wilt-inducing substance. Tomato plants wilted in culture filtrates did not recover when placed in distilled water. Sections measuring $\frac{1}{2}$ in. or more were cut under water from the basal part of stems of wilted cuttings and the plants were placed in distilled water. The plants completely recovered from their wilted condition within 2 to 10 hr. Plants wilted in dialyzed or nondialyzed filtrates or in aqueous solutions of the alcohol-insoluble precipitate from dialyzed filtrates behaved alike. The wilt produced by these solutions affected primarily the stems of the cuttings. These results suggested that the substance possibly causes wilt by plugging the vessels of the stem.

DISCUSSION

The evidence presented indicates that at least one of the wilt-inducing substances present in the culture filtrates is a polysaccharide. The substance is either nondialyzable or very slowly dialyzable. This fact simplified the partial purification since substances of smaller molecular size were easily removed. It is possible that there are other dialyzable, wilt-inducing

substances present in such filtrates. Although no attempt was made to compare directly the wilt-inducing activity of filtrates before and after dialysis, the results indicated that the nondialyzable substance accounted for a large part of the activity of the culture filtrates. Dialyzed and nondialyzed filtrates produced a similar type of wilt.

The thermal inactivation of nondialyzed, alkaline filtrates was interpreted incorrectly at first as indicating that the toxic substance was an enzyme or protein. It was shown that the polysaccharide obtained from dialyzed filtrates by alcoholic precipitation also was inactivated by heat when dissolved in an alkaline Richard's solution. The inactivation appeared to be due to removal of the polysaccharide from solution along with phosphates and other substances precipitated by heating the alkaline nutrient solution. The inactivation was not thought to be due to lowering the osmotic concentration by precipitation of the phosphates, since aqueous solutions of the polysaccharide were active.

Hodgson, Peterson, and Riker (9, 10) reported wilt-inducing polysaccharides from a number of organisms not associated with wilt diseases. They found the type of wilt produced was correlated with molecular size and solubility. Results of other experiments indicated that the wilting mechanism may be mostly physical (11). In the present study the toxic substance was found to cause wilting primarily of the stems and petioles of tomato cuttings. Since wilted cuttings recovered when placed in distilled water if sections $\frac{1}{2}$ in. or more in length were cut from the basal part of the stem, it appeared that the wilting was possibly caused by a physical blocking in the base of the cut stem.

Whether or not wilt-inducing substances present in culture filtrates of wilt organisms are involved in the production of wilt in naturally infected plants is not known. With a knowledge of the substances which may be involved, further investigations with naturally infected plants may lead to more definite conclusions.

SUMMARY

A wilt-inducing substance produced in liquid cultures of *Fusarium solani* f. *eumartii* was found to be nondialyzable, not filterable through bacterial filters, soluble in water, and relatively insoluble in several organic solvents.

The substance was precipitated from nondialyzed culture filtrates by the addition of two volumes of ethyl alcohol. Precipitation with alcohol from dialyzed filtrates was effected only after concentration of the filtrates.

The wilt-inducing substance was thermostable in neutral, aqueous solution and labile in strong acid solution. It was partially to completely inactivated by heat in nondialyzed, alkaline filtrates and in alkaline Richard's solution.

The toxic substance was found to induce wilting primarily in the stems and petioles of tomato cuttings. The leaflets generally were less affected.

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Wilting appeared to be caused by a condition brought about in the basal part of the cut stem, possibly a physical blocking. Wilted cuttings did not recover when placed in distilled water unless sections $\frac{1}{2}$ in. or more in length were cut from the basal part of the stem.

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CONTROL OF BLACK ROT IN WASHED, UNCURED SWEET POTATOES

W. J. Martin, J. M. Lutz, and G. B. Ramsey[1]

(Accepted for publication March 22, 1949)

Results of laboratory tests for the control of black rot caused by *Ceratostomella fimbriata* (Ell. and Hals.) Elliott in washed, uncured sweet potatoes[2,3] indicated that a momentary dip of the potatoes in a 1 per cent solution of borax would give adequate control of this disease. To further test the applicability of the borax-dip treatment, tests were made under commercial conditions. The purpose of this paper is to report the results of shipping and holding tests made in 1948 with borax-treated sweet potatoes and with sweet potatoes treated with an emulsion wax containing a fungicide, 2 per cent Dowicide A (sodium orthophenylphenate). The latter treatment was being used by several shippers who believed it was effective in controlling black rot.

Individual growers' lots of sweet potatoes containing black-rot lesions were selected for the tests. In preparing the test crates, the individual roots with visible lesions were graded out and were not included in the packed crates. Two 50-lb. crates, one treated and one untreated, were packed from each grower's lot. A total of 13 lots was used in the borax tests and 10 additional lots were used in the wax-fungicide tests. The experimental crates were packed at commercial packing sheds in Southwest Louisiana.

The tests with the borax treatment were made at a packing shed equipped with a dip tank. In this installation the sweet potatoes were washed by means of an overhead spray system and carried on chain rollers into the dip tank containing a solution of approximately 1 per cent borax. Another system of chain rollers carried them out of the tank to a conveyor belt, from which they were packed into the paper-lined commercial crates for shipment.

Tests with the emulsion wax containing a fungicide were made at two packing sheds equipped with foam waxers. The wax was applied to the sweet potatoes immediately after washing and just before packing, at the rate of 3 to 5 gal. per carload (504 bu.).

The experimental crates from six lots in the borax test and six lots in the wax-fungicide test were shipped to Chicago in commercial truck- or car-lots. Notes on the percentage of black rot in the treated and untreated

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[3] Lutz, J. M. Unpublished results obtained in 1946 and 1947.

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TABLE 1.—*Percentages of sweet potatoes that developed black rot in treated and untreated crates from 12 lots of sweet potatoes shipped to Chicago in August and September, 1948*

Lot number[a] and treatment	Percentages of sweet potatoes with black-rot lesions			
	On arrival		1 wk. after arrival[b]	
	Treated	Untreated	Treated	Untreated
Borax treatment				
1	0.0	0.0	0.0	0.0
2	0.0	0.0	0.0	0.0
3	0.0	0.7	0.0	0.7
4	0.0	0.0	1.0	0.0
5	0.5	0.0	2.1	4.0
6	3.7	0.6	3.7	5.0
Wax-fungicide treatment				
7	1.4	2.4	20.5	34.6
8	0.8	1.3	39.8	14.2
9	0.0	2.2	27.7	42.0
10	0.0	0.0	0.0	0.0
11	0.0	0.0	0.0	0.0
12	0.0	0.0	0.0	0.0

[a] Designating different crops.
[b] Includes lesions that developed in transit.

crates were recorded, on arrival (about 3 days after packing), and 1 week after arrival (Table 1). While relatively little black rot developed in either the treated or untreated crates of the borax test, considerable black rot developed, particularly after arrival, in both the treated and untreated crates from three lots in the wax-fungicide test. It can be noted that borax-treated lot number 6 had 3.7 per cent black rot on arrival but there was no increase

TABLE 2.—*Percentages of sweet potatoes that developed black rot in treated and untreated crates from 11 lots of washed, uncured sweet potatoes stored for 10 days in Southwest Louisiana in 1948*

Lot number[a] and treatment	Percentage with black rot	
	Treated	Untreated
Borax treatment		
1	0.0	12.9
2	0.0	1.2
3	0.0	11.9
4	0.0	14.8
5	0.0	0.0
6	0.0	0.0
7	0.0	10.7
Averages	0.0	8.0
Wax-fungicide treatment		
8	47.9	5.9
9	91.1	91.2
10	80.7	87.2
11	82.4	90.7
Averages	76.1	68.3

[a] Designating different crops.

during a 1-wk. holding period after arrival. It is probable that a slight infection at time of shipping would account for this.

Paired experimental crates from seven other lots that were treated with borax and from four lots receiving the wax-fungicide treatment were held in storage locally. Each lot was from a different crop. Notes on the percentages of black rot in these were recorded 10 days after packing (Table 2). Considerably more black rot developed in the lots held locally than in those that were shipped to Chicago. However, no black rot was found in any of the borax-treated crates, although an average of 8 per cent of the potatoes in the comparable untreated crates developed black rot lesions. Abundant black rot developed in the four lots in the wax-fungicide test, and the treated and untreated crates were equally affected.

No evidence of injury was noted in the borax-treated sweet potatoes. A darkening of bruised areas was noted in some cases on those given the wax-fungicide treatment.

The results of these tests indicate the effectiveness of the borax-dip treatment for the control of black rot in washed, uncured sweet potatoes, and the ineffectiveness of the emulsion wax containing sodium orthophenylphenate.

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SOIL TREATMENTS WITH CHLOROPICRIN, D–D, AND URAMON FOR CONTROL OF THE ROOT–KNOT NEMATODE[1]

C. N. Clayton and D. E. Ellis[2]

(Accepted for publication March 31, 1949)

Several chemicals such as chloropicrin, ethylene dibromide, and a mixture of dichloropropane and dichloropropene are known to be effective in control of *Heterodera marioni* (Cornu) Goodey (1, 4, 5, 6). Nevertheless, many questions regarding the use of such materials remain unanswered (1, 3, 5). One of these questions deals with the time of application. It is known that a certain period of time must elapse following the application of many soil treatment materials before crops can be planted without risk of injury (1, 5). However, it is not always feasible or practical to apply a treatment at a certain season of the year. Experiments herein reported were conducted to determine the effect of applying certain chemical nematocides to soil in spring, summer, and fall of one year upon the control of root-knot and upon the growth and yield of snap bean and tomato planted 14, 10, and 7 months after treatment and of cantaloupes planted 26, 22, and 19 months after treatment.

MATERIALS AND METHODS

The nematocides[3] used and the rates of application in pounds per acre were 1) Uramon, 2420; 2) Uramon, 4840; 3) D-D, 200; 4) D-D, 400; 5) D-D, 600; and 6) chloropicrin, 400. Nontreated plots were included as controls. Three replicates of each of the six treatments and the nontreated control were used for each of the three dates of treatment, March 11, July 12, and October 12, 1946, at each of 2 locations. The plot size was 6 by 12 ft. with 1-ft. alleys between plots. The soil type at location 1 (McCullers, N. C.) was Ruston sandy loam and at location 2 (Eagle Springs, N. C.) Norfolk fine sand. The soil at both locations was infertile and low in organic matter. The root-knot nematode population at McCullers was very high and uniform in distribution, whereas at Eagle Springs it was only moderately high and irregularly distributed.

The Uramon was broadcast uniformly by hand just prior to spading the soil about 6 in. deep. Using a hand applicator the D-D and chloropicrin were injected at 12-in. intervals and 5 in. deep into the soil. Im-

[1] Contribution from the Plant Pathology Section, Department of Botany, North Carolina Agricultural Experiment Station, Raleigh, North Carolina. Published with the approval of the Director as Paper No. 301 of the Journal Series.

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The assistance of R. G. Owens and R. S. Cox is gratefully acknowledged.

[3] The following commercial materials were furnished by the manufacturers: Larvacide (chloropicrin) by Innis, Speiden & Co.; D-D (1,3-dichloropropene and 1,2-dichloropropane mixture) by the Shell Chemical Corp.; and Uramon (urea) by the Ammonia Dept., E. I. du Pont de Nemours & Co.

mediately after injection of chloropicrin the surface of the soil was sprinkled with water in order to seal the fumigant in the soil.

Following treatment, weeds were permitted to grow on the plots until they were prepared for planting in the spring of 1947. On the chloropicrin-treated plots crab grass was the predominating species and it grew very well. Although the population of weeds and grass on the Uramon-

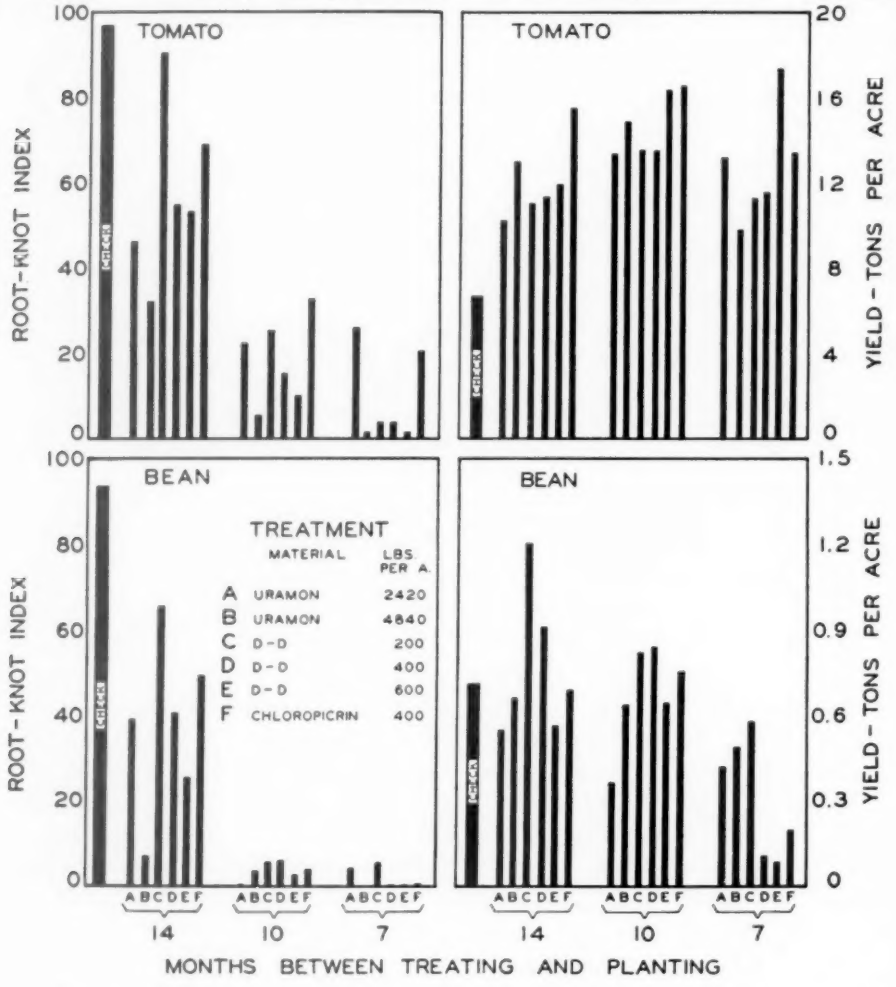

FIG. 1. Effect of soil treatments applied 14, 10, and 7 months prior to planting upon control of root-knot and upon yields of tomato and snap bean.

treated plots was greatly reduced, the plants that grew were strongly stimulated by the nitrogen from the Uramon. All plots were fertilized uniformly with 5–10–5 fertilizer at the rate of 2000 lb. an acre prior to planting.

Six Marglobe tomato plants grown in 2½-in. clay pots under glass and 100 Tendergreen snap bean seeds were planted on each plot in May, 1947.

Stand counts of beans were taken and ratings on the plant vigor of beans and tomatoes were made independently by three persons several times during the season. The yields of marketable green beans and tomatoes were taken. After harvest the roots of each plant were examined for root-knot. The bean plants were examined on July 29 and the tomatoes on August 18. The relative severity of root-knot was rated and indexes (2) for the different plots were calculated. The data from the 2 locations were generally in agreement. However, only the data for the McCullers test are presented here because at that location the infestation of the root-knot nematode was distributed uniformly. The data were analyzed by the Department of Experimental Statistics, North Carolina State College. Only statistically significant data are stressed.

RESULTS

Effect on root-knot control. The data on root-knot control (Fig. 1) show that all treatments applied in October (7 months prior to planting) reduced root-knot to a very low level in comparison with the nontreated. For most treatments applied in July (10 months before planting) the average root-knot indexes, particularly for tomato, were somewhat higher than for the respective treatments applied in October. The indexes for the treatments made in March (14 months prior to planting) were much higher than those for treatments applied in July or October. This higher incidence of root-knot on the plots treated in March was most likely the result of a large increase in the population of the root-knot nematode during the summer months following treatment. The data in figure 1 indicate that the higher rates of D-D and of Uramon were generally more effective than the lower rate.

Effect on yields. All materials at all dates of application reduced the nematode infestation sufficiently to permit much better growth and yield of tomatoes than on nontreated plots. Although the degree of root-knot control on beans was similar to and somewhat better than that on tomatoes, this was not generally reflected in increased yields. In fact, bean yields from all plots treated 7 months before planting were lower than those from the nontreated, indicating that some deleterious effect on growth of beans resulted from some of the treatments. This was somewhat more noticeable on plots treated with chloropicrin and the higher rates of D-D seven months before planting than on those treated 10 or 14 months prior to planting (Fig. 1). This deleterious effect and the control of root-knot were converse factors affecting the growth of beans. This explains why the highest yields occurred on plots which were treated with D-D at the lowest rate and yet had the highest root-knot index. The yield data were closely paralleled and substantiated by those on plant vigor. Therefore the latter are not given.

Effect of water-seal with D-D. Keeping the soil surface wet for a few days after chloropicrin has been injected has been found (4) to increase

the effectiveness of the fumigant by sealing it in the soil until the nematodes are killed. In conjunction with the preceding experiment, D-D at

TABLE 1.—*Comparative effectiveness of D-D applied at 200 lb. and 400 lb. per acre with or without a water-seal 14, 10, and 7 months prior to planting, upon root-knot control and upon yield of tomato and snap bean*

Treatment			Time of application in 1946	Root-knot index		Yield per acre	
Material	Rate of application per acre	Water-seal		Tomato	Bean	Tomato	Bean
	Lb.					*Tons*	*Tons*
D-D	200	No	March	91	66	11.0	1.20
			July	25	5	13.6	0.82
			October	4	6	11.3	0.57
D-D	200	Yes	March	79	36	16.7	1.24
			July	16	22	14.8	0.75
			October	4	2	13.2	0.47
D-D	400	No	March	55	41	11.3	0.91
			July	11	6	13.6	0.85
			October	4	1	11.6	0.10
D-D	400	Yes	March	37	15	15.0	1.06
			July	16	6	14.1	0.52
			October	0	1	11.9	0.13
None (check)	.....	.....	.....	97	94	6.5	0.71

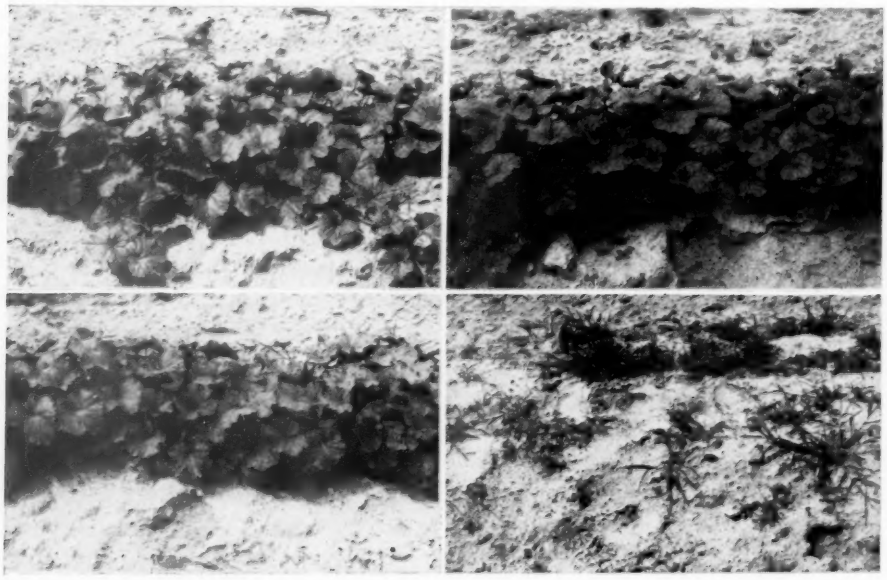

FIG. 2. Cantaloupe plants in 1948 on plots treated on October 12, 1946. Upper left, chloropicrin, 400 lb. per acre, plus a water seal. Upper right, D-D, 400 lb. per acre. Lower left, Uramon, 2420 lb. per acre. Lower right, no treatment (plants killed by root-knot).

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200 and 400 lb. per acre with and without a water-seal was applied 14, 10, and 7 months prior to planting of tomato and snap bean. The results (Table 1) of these tests at McCullers indicate that root-knot was well controlled with both dosages of D-D applied 7 or 10 months prior to planting and with or without a water-seal. The water-seal improved the control only when used in the March treatments, 14 months prior to planting.

The bean yields appeared not to be significantly affected by water-seal. The plots treated with D-D at 200 lb. outyielded those treated at the 400-

TABLE 2.—*Effect of soil treatments applied on March 11, July 12, and October 12, 1946 upon root-knot control and upon yield of cantaloupes in 1948*

Treatment			Time of application in 1946	Root-knot index	Yield of cantaloupes per plot (number)
Material	Rate of application per acre (in lbs.)	Water-seal			
Uramon	2420	No	March	81	4
			July	41	17
			October	29	13
Uramon	4840	No	March	85	9
			July	19	17
			October	46	13
D-D	200	No	March	100	5
			July	69	12
			October	70	16
D-D	200	Yes	March	100	6
			July	88	17
			October	34	33
D-D	400	No	March	95	6
			July	86	20
			October	44	23
D-D	400	Yes	March	81	17
			July	74	19
			October	15	26
D-D	600	No	March	91	4
			July	63	23
			October	8	18
Chloropicrin	400	Yes	March	95	13
			July	87	14
			October	71	12
None (check)	.....	.....	.....	100	3

lb. rate. The effect of the latter in the October application was to reduce drastically the yield to about 1/8 that of the March treatment and about 1/7 that of the nontreated. These low yields are not attributable to root-knot. There was a highly significant difference in yields of beans for the three dates of treatment.

Tomato yields from plots treated 7 or 10 months prior to planting were not affected by water-seal, while, on those treated 14 months ahead of planting, a water-seal resulted in a significant increase in yield. The yield of marketable tomatoes on nontreated plots in 1947 was approxi-

mately ½ that on plots treated with D-D at 200- or 400-lb. per acre with or without a water-seal.

Effect upon root-knot and yield of cantaloupes planted second year after treatment. Cantaloupes were planted in 1948 on the plots treated in March, July, and October of 1946 and cropped to tomatoes and beans in 1947. Because of less injury from root-knot even in the second year after treatment, the stand and growth of plants on treated plots were much better than on nontreated plots (Fig. 2). Furthermore, the data on control of root-knot and on yield (Table 2) show that the effect of treatment persisted into the second year, although it was less pronounced than in the first year after treatment. In general, the treatments which were the more effective against root-knot in 1947 continued to be the more effective ones in 1948.

All treatments resulted in increased yields of cantaloupes in comparison with the nontreated. The yields from all July or October treatments were 4 to 10 times greater than from the check. However, the increases from the treatments applied in March 1946 were relatively small except with chloropicrin or with D-D at 400 lb. plus a water-seal.

SUMMARY

1. Applications of chloropicrin at 400 lb.; D-D at 200, 400, and 600 lb.; and Uramon at 2420 and 4840 lb. per acre were made to sandy loam soil in March, July, and October of 1946 prior to the planting of tomatoes and snap beans in 1947 and cantaloupes in 1948.

2. In comparison with the nontreated checks, all October and July treatments and some of those applied in March effectively reduced root-knot. In general, the treatments applied in July or in October resulted in better control than those applied in March.

3. In the March application, Uramon at 4840 lb. per acre was more effective in controlling root-knot than at 2420 lb., while D-D at 600 or 400 lb. per acre gave better root-knot control than at the 200-lb. rate.

4. In comparison with the nontreated check, all treatments resulted in much better growth and higher yields of tomatoes. Similar results were not obtained with beans although root-knot control with the two crops was similar.

5. Some toxic or deleterious effect on growth and yield of beans resulted from treatment with D-D, chloropicrin, or Uramon.

6. Root-knot was well controlled with D-D applied 7 or 10 months prior to planting at rates of 200 and 400 lb. per acre with or without a water-seal.

7. The beneficial effect of treatment persisted into the second year and was reflected in improved growth and yield.

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EFFECTS OF SOIL TREATMENTS WITH URAMON AND CERTAIN FUMIGANTS UPON PLANT GROWTH AND INCIDENCE OF ROOT-KNOT[1]

D. E. Ellis, C. N. Clayton, and R. G. Owens[2]

(Accepted for publication March 31, 1949)

Root-knot, caused by *Heterodera marioni* (Cornu) Goodey, is one of the most serious diseases affecting southern vegetable crops. In North Carolina it is a particularly important problem in home gardens and frequently causes heavy damage in commercial plantings. The disease is also an important factor in the establishment of new peach orchards. While control of root-knot by crop rotation is fairly effective (4), the method is often not applicable to the above crops. Many chemical soil treatments have shown promise against root-knot (1, 2, 8, 10), but many questions regarding their use are as yet unanswered (7, 12). Field studies were made to obtain more information on the effects of rate and time of application of various materials upon root-knot control and the response of various plants, on possible cumulative toxicity to plants resulting from repeated applications in successive years, and on methods of reducing injury to crops following treatment with Uramon.

MATERIALS AND METHODS

For the tests outlined below the nematocidal materials were supplied by the manufacturers.[3] Uramon, Cyanamid (calcium cyanamide), peanut-hull meal, cottonseed meal, and manure were broadcast evenly by hand and immediately disked or spaded into the soil to a depth of about 6 in. Rye was sown broadcast by hand, covered with a disk or hand rake, and the resulting crop disked or spaded into the soil about a month in advance of planting. The volatile fumigants were injected into the soil at 12-in. intervals and 5 in. deep with a hand injector. A water seal was used on all chloropicrin plots. All materials were tested in the experiments at McCullers, North Carolina. One experiment was duplicated at Eagle Springs, North Carolina. In all tests care was taken to prevent transfer of soil from one plot to another by providing adequate borders and drainage furrows between plots, and by using care in cultivation, most of which was done by hand. All plots received equal applications of commercial

[1] Contribution from the Plant Pathology Section, Department of Botany, North Carolina Agricultural Experiment Station, Raleigh, North Carolina. Published with the approval of the Director as Paper No. 302 of the Journal Series.

The assistance of R. S. Cox is gratefully acknowledged.

[2] Respectively, Associate Professor, Associate Professor, and Assistant, Plant Pathology Section, Department of Botany.

[3] Larvacide (chloropierin), Iscobrome D (ethylene dibromide 10 per cent by volume), and ethylene chlorobromide by Innis, Speiden & Co.; D-D (1–3 dichloropropene and 1–2 dichloropropane mixture) by the Shell Chemical Corporation; Dowfume W-10 (ethylene dibromide 10 per cent by volume) by the Dow Chemical Company; and Uramon (urea) by the Ammonia Department, E. I. du Pont de Nemours & Company.

5–10–5 or 6–8–6 fertilizer at rates of 1500 to 2000 lb. per acre applied in the row or broadcast by hand from 2 to 7 days prior to planting.

The plants used in the various tests included Tendergreen snap bean, Marglobe tomato, Clemson Spineless okra, and Lovell peach. The tomato plants were started in steam-disinfested soil under glass. Stand counts, growth measurements, and yields of the three vegetable crops were obtained. In certain tests a further measure of vegetative growth was obtained by weighing the green plants after harvest was complete. At the end of the harvest period the plants were dug, the roots rated for root-knot, and root-knot indexes (6) calculated. The data were analyzed by the Department of Experimental Statistics, North Carolina State College.

SOIL TREATMENTS WITH URAMON

Although soil treatment with Uramon is known to be highly effective in control of root-knot (3, 5, 7, 11), its use often results in stunted plants

TABLE 1.—*Effect of soil treatment with Uramon and certain supplementary materials upon control of root-knot and upon yield of tomatoes*

Treatment[a]	Root-knot index		Yield of tomatoes (tons per acre)
Materials and rates (lb. per sq. yd.)	1946	1947	1946
Uramon 1	8.7	28.7	5.2
Do + Cyanamid ½	11.8	36.6	4.0
Do + Peanut-hull meal 1	10.6	52.5	7.7
Do + Cottonseed meal 1	13.7	35.4	9.1
Nontreated (check)	98.5	100.0	3.1
L.S.D. (0.05)	18.9	25.5	2.1

[a] The soil was Norfolk sandy loam heavily infested with root-knot nematodes. Four replicate plots, 0.004 acre each, were used for each treatment. The Uramon and Cyanamid were applied October 29, 1945, and the other materials on February 25, 1946. Tomato plants were set on the plots on May 8, 1946, and May 5, 1947.

and low yields in the first crop after treatment (3, 7, 11). The addition of certain organic materials to counteract such injury has shown promise (7, 9). Results of a preliminary test in 1945 indicated that the application of finely ground peanut hulls or cottonseed meal to Uramon-treated soil was somewhat effective against the deleterious effects of this treatment on the first crop after treatment (7).

Table 1 shows the results of an experiment in which Uramon plus Cyanamid, Uramon plus peanut-hull meal, and Uramon plus cottonseed meal were applied in the fall and winter of 1945–46 prior to planting crops in the spring of 1946 and 1947. Although all treatments effectively reduced the incidence of root-knot in 1946 in comparison with the check, there were no significant differences between the root-knot indexes for the treated plots. This was still the case in 1947, although on the treated plots there was considerable increase in root-knot in comparison with the

respective indexes for 1946. The average yield of tomatoes in 1946 from plots treated with Uramon plus peanut-hull meal and Uramon plus cottonseed meal were 2.5 to 3 times that from the nontreated plots.

The phytotoxic effect of the Uramon treatment was found to vary with different soil types in other experiments (3). The effectiveness and phytotoxicity of treatments with Uramon plus several supplements was further studied on Norfolk sandy loam soil very heavily infested with root-knot nematodes at McCullers, North Carolina, and on Norfolk fine sand practically free from root-knot nematodes at Eagle Springs, North Carolina. The treatments and subsequent cropping history were similar at both locations.

The results of the experiment at McCullers are shown in table 2.

TABLE 2.—*Effect of Uramon plus several supplementary treatments upon control of root-knot and yields of tomato, okra, and snap bean at McCullers, North Carolina*

Treatment[a]	Average root-knot index	Av. yields per acre		
Materials and rates (lb. per sq. yd.)		Tomato (tons)	Okra (bu.)	Bean (bu.)
1. Uramon ½	47	4.83	173	90
2. Do ¾	55	4.59	202	90
3. Do ½ + Rye cover crop	49	7.59	226	94
4. Do ½ + do	35	5.94	239	104
5. Do ½ + Peanut-hull meal 1	56	5.34	202	67
6. Do ½ + do	37	5.31	208	69
7. Do ½ + Manure 4	56	5.01	193	65
8. Untreated check	87	3.75	82	82
L.S.D. (0.05)	16	1.40	77	20

[a] Four replicate plots, 0.007 acre each, were used for each treatment. The Uramon was applied on October 1, 1947, and the other materials were applied and the rye sowed on October 30, 1947. Peach pits and 3 peach seedlings, 7 tomato plants, 100 bean and 200 okra seeds were planted on each plot in the spring of 1948. Low soil moisture from May to July reduced and retarded the development of root-knot and of snap bean.

The root-knot indexes for okra, tomato, and peach are averaged since they showed similar trends. While all treatments significantly reduced root-knot, in comparison with the nontreated check, none reduced it to a very low level. However, yields of tomato and particularly of okra showed beneficial effects from most treatments in comparison with the check. The highest yield of bean, of okra, and of tomato was obtained on Uramon plus rye plots. Despite the fact that Uramon at the lowest rate permitted root-knot incidence to be fairly high, the treatment reduced it sufficiently to allow good growth of okra and peach whereas the plants in the nontreated plots were stunted and dead (Fig. 1). This increase in growth is believed to be primarily the result of root-knot control. The plants on nontreated plots, where root-knot was severe, were stunted and killed prematurely but, in the experiment at Eagle Springs, where root-knot was definitely not a factor, the best growth occurred on the nontreated plots (Fig. 2).

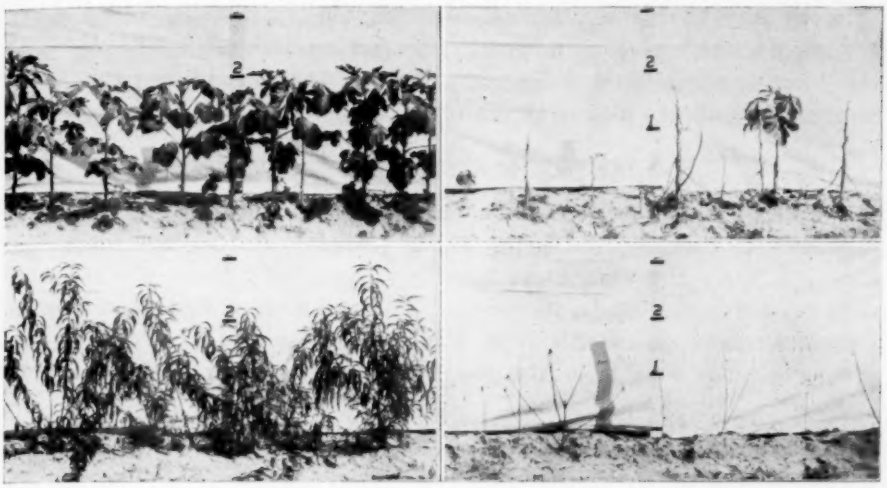

FIG. 1. Okra and peach plants on plots treated with Uramon, ¼ lb. per sq. yd., on October 1, 1947, followed by a winter cover crop of rye (left), in comparison with those killed by root-knot on nontreated plots (right). Photographed August 27, 1948. McCullers, North Carolina.

The results at Eagle Springs are summarized briefly. The root-knot indexes were practically zero for all treatments and the nontreated checks with all crops. The growth and yields of the beans were similar for the various treatments. The yields of tomatoes appeared to be affected very

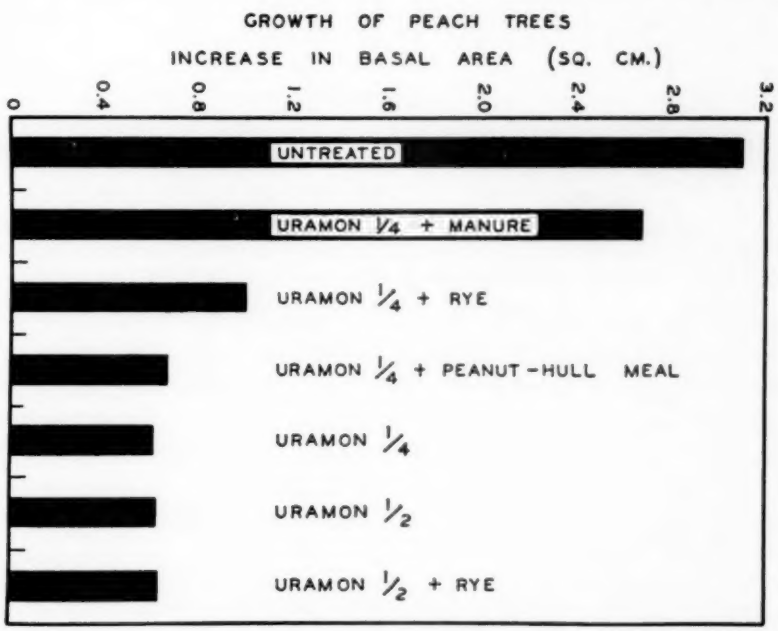

FIG. 2. Growth of peach trees in first season following soil applications of Uramon at ¼ and ½ lb. per sq. yd. on Oct. 1, 1947, and of addition of supplements to Uramon-treated plots on November 1, 1947. Eagle Springs, North Carolina.

little by any of the treatments. The phytotoxic effect of the Uramon treatments was severe upon peach trees set on December 23, 1947. At this location the addition of manure was the only amendment that counteracted this effect on peach trees (Fig. 2).

SOIL TREATMENTS WITH VOLATILE FUMIGANTS

Rapid strides have been made in the development and use of volatile chemicals to disinfest soil prior to the planting of root-knot-susceptible crops (2, 10). However, many questions regarding the use of such materials remain inadequately answered (7, 12). Although fumigants applied at commercially practicable rates will reduce root-knot, they will neither eradicate nematodes from the soil nor prevent their re-entry (2, 10).

TABLE 3.—*Effect of soil treatment with volatile fumigants applied in 3 successive years upon root-knot control and yields of tomato and snap beans*

Treatment[a]	Average root-knot index			Average yield (bushels per acre)			
				Tomato		Snap bean	
	1946	1947	1948	1947	1948	1947	1948
Chloropicrin	0.2	1.0	0.1	446	197	22	24
D-D	1.5	9.9	9.1	540	203	41	61
Ethylene chlorobromide	0.1	2.5	0.2	541	203	140	68
Ethylene dibromide (Dowfume W-10)	3.3	11.3	2.8	511	210	103	98
Ethylene dibromide (Iscobrome D)	10.0	4.6	1.4	422	313	87	67
Untreated (check)	71.3	95.5	70.0	254	110	90	71
L.S.D. (0.05)	9.6	9.2	8.5	147	91	45	N.S.

[a] All materials were applied at a rate of 2.6 ml. per sq. ft. This rate expressed on an acre basis is approximately 400 lb. of chloropicrin and ethylene chlorobromide, 300 lb. of D-D, and 220 lb. of Dowfume W-10 and Iscobrome D. The dates of application were May 16–20, 1946; May 2, 1947; and April 21, 1948. The soil was Ruston sandy loam uniformly and moderately infested with root-knot nematodes. Two replicate plots, 0.003 acre each, were used for each treatment. Beans and tomatoes were planted about 1 month after treatment in each of the 3 years.

Therefore, for continuous control the treatment must be repeated. Little information is available on possible cumulative deleterious effects of successive treatments applied to the same soil. Furthermore, additional information is needed regarding the response of various crop plants to fumigants applied at different rates and seasons. While certain fumigants effectively control root-knot, this benefit is sometimes nullified by injury. Recently it has been shown (3) that while certain nematocides reduce root-knot to about the same level on bean and tomato, the response of the two crops is vastly different. Also, there were marked differences in control of root-knot and in plant growth and yield of crops following treatment in the spring, summer, or fall. The objectives of the following experiments were to throw more light upon these problems.

Treatment in successive years. Results from the use of certain volatile fumigants applied to the same plots in 3 successive years are given in table 3. The average root-knot indexes show that all treatments reduced root-knot to a low level. However, in all plots the disease was present, at

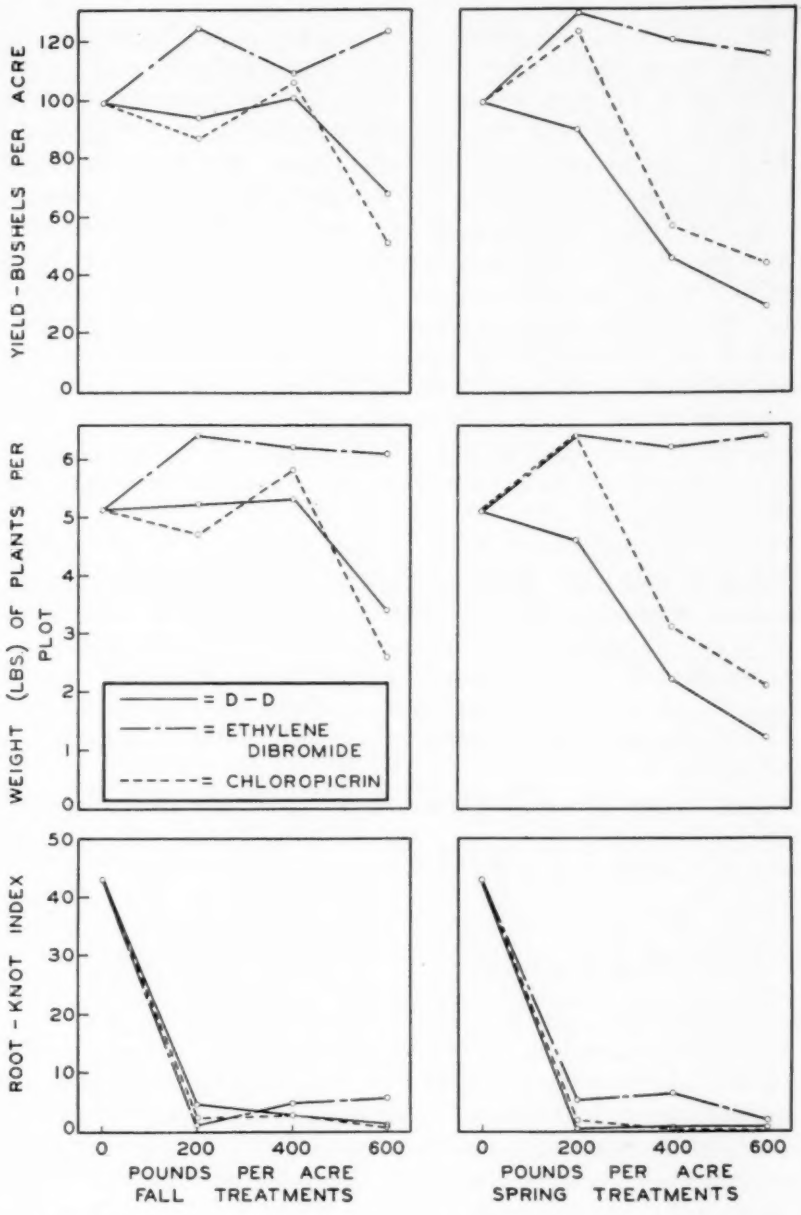

FIG. 3. Effect of soil treatment with certain fumigants applied at different rates in fall and in spring prior to planting of snap beans, upon root-knot, yield, and weight of green plants.

least in trace amounts, in the third year. After the first year all treatments markedly increased yields of tomatoes. Although yields fluctuated considerably from year to year, there was no clear-cut indication of cumulative phytotoxic effects. There was, however, an indication that the chloropicrin and D-D treatments depressed the growth and yield of beans.

Rate and time of application. Fall and spring applications of chloropicrin, D-D, and ethylene dibromide (Iscobrome D), each at rates of 200, 400, and 600 lb. per acre, were compared. The soil (Norfolk sandy loam) was irregularly and only lightly to moderately infested with root-knot nematodes. The plot size was 0.003-acre and each treatment was replicated 4 times in randomized blocks. The fall applications were made on October 3, 1947 (soil temperature 65° F.), and the spring applications were made on March 26, 1948 (soil temperature 63° F.). During the 5 months following the fall application the soil moisture was at abnormally high levels. On each plot 200 bean seeds (100 in each of 2 rows) were planted on May 6. The bean seeds for planting one row were inoculated with the proper nodule-forming bacteria for snap bean; those for the other row were not inoculated. Since no noticeable differences in stand, vigor, yield, or nodulation could later be detected, the data for inoculated and noninoculated bean rows were combined. The bean plants were examined for root-knot in July.

The results presented in figure 3 show that while all three materials at each rate applied in spring or fall reduced root-knot to a very low level, some treatments markedly depressed growth and yield. The data on yield and on green weight of plants show that injury resulted from treatment with D-D and chloropicrin, applied at the highest rate in the fall and at the two higher rates in the spring. The injury was greatest at the highest rate. Treatments with ethylene dibromide appeared to be noninjurious.

Yields of beans on plots treated with D-D at 200 lb. per acre in the spring were significantly lower than on those treated with ethylene dibromide or chloropicrin at the same time and rate.

These data confirm previous work (3) showing that date of treatment, dosage, and response of certain crops are important factors to be considered in soil fumigation.

SUMMARY

1. In field experiments, root-knot was effectively reduced and yields of tomato were markedly increased by soil treatment with Uramon, chloropicrin, D-D, ethylene dibromide, or ethylene chlorobromide. Annual applications of the latter four materials in three successive years to the same plots neither eradicated the root-knot nematode nor resulted in any noticeable cumulative phytotoxicity.

2. Uramon, at rates as low as ¼ lb. per sq. yd., applied to light sandy soil heavily infested with *Heterodera marioni* in the fall prior to planting

in the following spring, usually resulted in increased growth and yields in comparison with those from nontreated plots. These increases were large enough to offset the residual deleterious effect of the Uramon treatment. In some experiments the addition of peanut-hull meal, cottonseed meal, manure, or a rye cover crop to plots treated with Uramon counteracted this deleterious effect upon certain crops.

3. On soil lightly infested with the root-knot nematode, applications of ethylene dibromide at 200, 400, and 600 lb. per acre in the fall or spring, of D-D and chloropicrin at 200 and 400 lb. in the fall or of chloropicrin at 200 lb. in the spring appeared to be noninjurious to snap bean. D-D and chloropicrin applied in the fall at 600 lb. or in the spring at 400 and 600 lb. per acre resulted in marked reductions in yield and growth of snap bean.

PLANT PATHOLOGY SECTION
NORTH CAROLINA AGRICULTURAL EXPERIMENT STATION
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PHYTOPATHOLOGICAL NOTES

A Witch's-Broom Disease on Cucumber and Canteloupe.—During the latter part of the recent war the author was employed by the Foreign Economic Administration in connection with its program of vegetable production for the armed forces on certain of the islands in the South Pacific area. On Espiritu Santo in the New Hebrides group an unusual and interesting disease,[1] presumably caused by a virus, was observed on plantings of canteloupe (*Cucumis Melo*) var. P.M.R. No. 45, and cucumber (*C. sativus*) var. Long Green. So far as can be determined this disease has not been previously described. Circumstances did not permit experimenting, so that only a description of symptoms was obtained. The symptoms described are those observed on cucumber; those on canteloupe were essentially the same.

General symptoms were of a "witch's-broom" type. The infected portions of the plants appeared as a mass made up of numerous flowers and undersized leaves. In early infection the whole plant was much reduced in size, showing typical symptoms throughout; in later infection only subsequent growth was affected. Mosaic and other pattern type symptoms were absent. The infected portions were uniformly green, slightly paler than normal, with spindly stems. Infected plants remained alive and growing until removed by cultural operations.

Internodes were somewhat shortened, and nodes in contact with moist soil readily produced adventitious roots. On growth subsequent to infection, branching occurred at every node of the primary stems. Every node on both primary and secondary stems produced from three to six flower buds, usually only one flower opening at a time. The shortened internodes, excessive branching with leaves at each node, and profuse flower production gave the characteristic dense "witch's-broom" appearance.

Leaves, though undersized, were nearly normal in shape. Petioles were seldom more than 2 in. long, blades not more than $2\frac{1}{2}$ in. long by $1\frac{1}{2}$ in. wide, often smaller. Flowers were abnormal and nonfunctional. Pedicels were 1 to $1\frac{1}{2}$ in. long, very slender and weak. Petals of open flowers were approximately $\frac{1}{2}$ normal size, yellow to greenish-yellow.

The proportion of male to female flowers was far in excess of the usual. Male flowers had definitely enlarged sepals which were either narrow and straplike, up to $\frac{1}{2}$ in. long, or more often leaflike with a definite petiole and lanceolate to pointed ovate blade up to $\frac{3}{8}$ by $\frac{1}{2}$ in. Female flowers had normal-shaped sepals proportionate in size to the petals. Ovaries at time of blossoming were about $\frac{3}{8}$ in. long, more slender than usual and with

[1] Early in March, 1949, Dr. O. A. Lorenz observed the same or a very similar disease causing extensive damage on commercial plantings of cucumbers near La Paz, Baja California, Mexico. The presence of a new disease of this type so close to production areas in the United States is noteworthy.

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spines much reduced. Sometimes developing to 1–1½ in., the ovaries always turned yellow and dropped, as did fruit partially grown before infection occurred.

No other cultivated cucurbits than cucumber and canteloupe were available for study during the period when the disease was observed. A *Momordica sp.* which grew profusely everywhere on the island may have been a symptomless carrier, but the definite seasonal occurrence noted below would be unlikely with an infected host in close proximity to the cultivated crops. An unidentified, much less common cucurbit which had similar symptoms may have been the source of the disease. Since the farming area was bordered on three sides by thick jungle growth, other host plants may have been present. The vector was not observed.

Although cucumbers were grown continuously throughout the year, the disease was observed only during December, January, and February, 1944–45. These summer months, on Espiritu Santo, are considerably less rainy than the rest of the year, and in this particular season virtually no rain fell during the months named, while the remainder of that year was extremely wet. Thus, considering the great variations in the island's rainfall during the year, together with the small annual range in mean temperature, it seems probable that the incidence of the disease is related to the former. The heavy rainfall during most of the year may have served to keep the presumed insect vector in check.—PAUL G. SMITH, Division of Truck Crops, University of California, Davis, California.

Asteroid Spot of Peach in North Carolina.—Leaves of three Indian Chief peach trees in a variety orchard of the Agricultural Experiment Station at Eagle Springs, North Carolina, had symptoms in July, 1946, resembling those of some virus diseases. These trees had been obtained from the Wolfe Nursery, Stephenville, Texas, and planted adjacent to each other in March, 1945.

Symptoms of the disease were similar in many respects to those of asteroid spot.[1] Affected leaves were rather uniformly stippled with numerous small, irregularly shaped, yellow or yellowish-green areas. Only leaf symptoms were noted. No breaking of the blossoms, delay in foliation, premature defoliation, reduction in growth, or malformation of leaves, fruit, or flowers have been observed. The leaf symptoms are imperceptible early in the season, but become rather conspicuous with the advent of high temperatures towards mid-season, and remain distinct on fully expanded leaves until they drop in the fall.

The disease has been found to be bud-transmissible. Buds from affected trees were inserted on September 6, 1946, into four J. H. Hale and one Golden Jubilee peach tree growing in 10-in. pots under glass. All buds united with the stocks but shoots grew from only three of them. The leaves

[1] Cochran, L. C., and Clayton O. Smith. Asteroid spot, a new virosis of the peach. Phytopath. 28: 278–281. 1938.

on these shoots and on branches below the point of budding had typical symptoms in June, 1947. On June 6, 1947, buds from affected Indian Chief trees were placed in seven potted Golden Jubilee trees. Leaf symptoms were apparent on shoots below the point of budding by August 21, 1947. In another test started in September, 1947, buds from affected Indian Chief trees were placed into 16 potted trees including one or more of 14 varieties. All buds united with the stocks. In 1948, shoots grew from six of the buds. Typical symptoms developed by midsummer on the leaves of these Indian Chief shoots. The leaves of other shoots on the 16 budded trees also showed typical symptoms, being more conspicuous for some varieties than for others. Figure 1 shows a J. H. Hale leaf from a branch growing above an

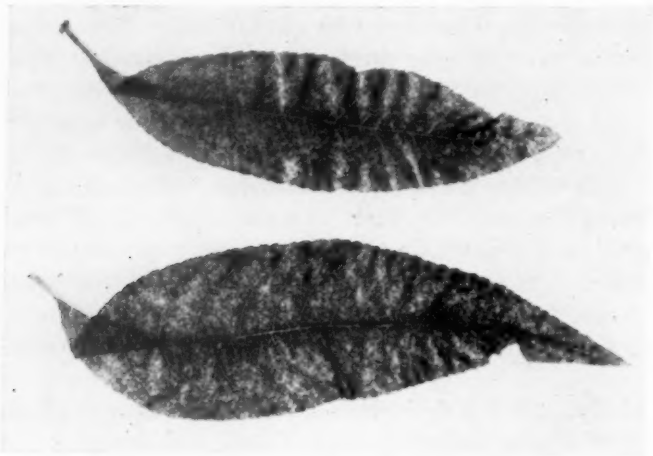

FIG. 1. Peach leaves with symptoms of asteroid spot. Upper, from a J. H. Hale shoot that grew above an inserted Indian Chief bud. Lower, an affected Indian Chief leaf.

inserted bud, in comparison with one that grew from an inserted Indian Chief bud. No symptoms appeared in the controls.

Examination of specimens and photographs of affected leaves kindly sent by L. C. Cochran[2] supports the evidence that the three Indian Chief peach trees are affected by the virus disease known as asteroid spot.—C. N. CLAYTON, Plant Pathology Section, Agricultural Experiment Station, Raleigh, North Carolina.

Leaf Distortion in Sunflower Produced by Lanolin.—Lanolin paste has been used in various scientific investigations involving introduction of exceedingly small amounts of chemicals into plant cells and tissues. Since the time of Laibach[1] plant physiologists have been using it in plant hormone work, and geneticists as well as horticulturists have used it in their

[1] Laibach, F. Versuche mit Wuchstoffpaste. Ber. Deut. Bot. Ges. 51: 386–392. 1933.

[2] Letter of November 18, 1948.

work in connection with the colchicine treatment of experimental materials. However, no report of any anatomical and morphological abnormalities attributable to lanolin itself in plants has been found by the writer.

Sunflower plants, of the variety Russian Mammoth, were grown in 6-in. pots in a warm greenhouse. When the first set of leaves was well de-

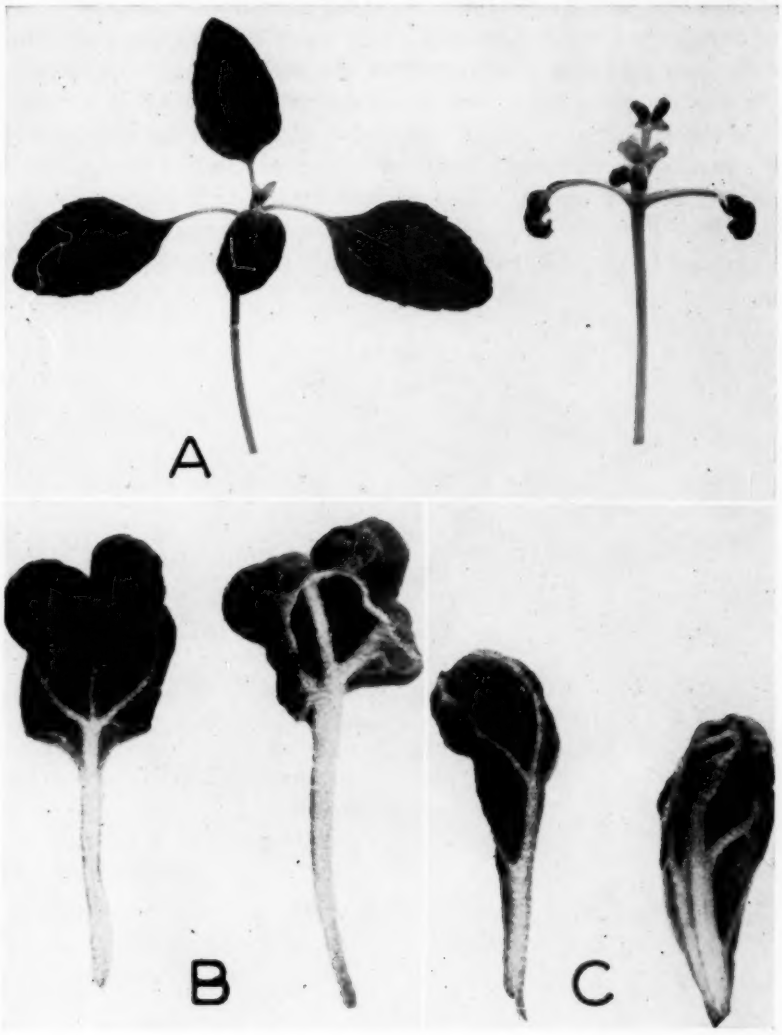

FIG. 1. Effect of lanolin on sunflower leaves. A. Left, check plant, treated with distilled water; right, plant with lanolin-treated cut surface. B, C. Dorsal and ventral views of the leaves from a lanolin-treated plant.

veloped, the growing point was removed with a sharp scalpel and a small amount of lanolin was placed on the cut surface with a glass rod. The lanolin used was U.S.P. grade, anhydrous, manufactured by Mallinckrodt Chemical Works. The checks consisted of sunflower plants with growing points removed, the wound treated with a drop of distilled water.

The plants were left on the bench in the same room. In a week or 10 days axial buds usually commenced to grow and were quite noticeable. On plants treated with a drop of water the leaves were normal while in lanolin-treated plants the leaves were underdeveloped and distorted in various ways (Fig. 1). This condition persisted for some time indicating a possible introduction of something into the plant from the lanolin. The morphological appearance of the sunflower leaves was somewhat like that of certain virus diseases and also was somewhat similar to injury by certain new herbicides such as 2,4-D. There is considerable reduction in growth as a result of the lanolin treatment. Distorted leaves develop very slowly and become brittle, breaking off easily on slight pressure. The phenomenon was not observed in tomato plants used at the same time in parallel experiments; hence it seems there is a certain degree of specificity.—PETER A. ARK, Division of Plant Pathology, University of California, Berkeley, California.

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